UNIVERSITY OF KWAZULU-NATAL

BREEDING MAIZE FOR RESISTANCE TO THE FALL ARMYWORM (*Spodoptera frugiperda* J.E. Smith), IMPROVED YIELD AND YIELD-RELATED TRAITS

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Breeding Maize for Tolerance to The Fall Armyworm (*Spodoptera frugiperda*), Improved Yield and Yield-related Traits

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THESIS ABSTRACT

Maize production and productivity in sub-Sub-Saharan Africa (SSA), including Zambia, has been severely threatened by the recent arrival of the fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith). Several strategies have been proposed to control FAW. Integrated pest management (IPM), emphasizing host-plant resistance, has been identified as the most sustainable approach. However, validated and locally-adapted FAW-resistant maize cultivars have not yet been developed and deployed in sub-Saharan Africa. The aim of this research was to develop FAW resistant, farmer-preferred and locally adapted maize varieties. The specific objectives of this study were: (1) to identify farmers' maize production constraints and preferred traits, and to assess farmers' perceptions of the impact of FAW on maize production and productivity in Zambia; (2) to screen a diverse population of maize genotypes for FAW resistance, yield, and yield-related traits by phenotyping and genotyping, to select the most promising genotypes for crossing; (3) to optimise the methods for laboratory rearing of FAW, and to conduct artificial screening of promising maize genotypes under controlled conditions and artificial FAW infestation; (4) to determine the level of genetic diversity for agronomic and FAW-related traits among the test population of maize genotypes using SNP markers complemented by phenotypic information to identify suitable parents for developing FAW-resistant breeding populations; and (5) to determine the nature of gene action conditioning FAW resistance, yield, and yield-related traits through combining ability analysis, and to identify the most promising crosses for continued evaluation in FAW resistance breeding.

In the first study, participatory rural appraisal (PRA) surveys were conducted in two FAW affected districts in Zambia in 2017 and 2018, using semi-structured questionnaires, preference ranking and focus group discussions. The high cost of fertilizers, the limited availability of agricultural lands, insect pests, and drought stress were reported by 73, 55, 38 and 36.6% of the respondents, respectively, as the main production constraints. There were significant differences (X^2 = 12.415; p = 0.002) in the severity of FAW infestation between the two surveyed districts in 2017. Farmer-preferred traits of maize were insect pest resistance, early maturity, drought tolerance and market price of the grain. FAW resistance, drought tolerance and grain yield performance are the key drivers for maize variety development and deployment in Zambia.

In the second study, two sets of diverse maize germplasm were assessed for FAW resistance and desirable yield-related traits in a FAW-affected area in Zambia. Set I and Set II, containing

60 and 253 maize genotypes, respectively, were selected, based on their agronomic potential and adaptability. Highly significant differences ($P < 0.001$) were detected among the test genotypes for FAW leaf damage (FLD), FAW cob damage (FCD) and agronomic traits. The lowest levels of FLD and FCD were 8.87% and 5.36%, recorded for genotypes CML304-B and CML442, respectively. Five principal components (PCs) accounted for ≥80% of the total variation associated with reduced anthesis-silking interval (ASI), plant height, FLD and FCD, desirable ear aspect and grain yield. Genotypes such as Pool 16 and ZM 7114 from Set I, and CZL1310c, CML444-B, CZL15220 and TL1512847 from Set II had low mean FCD and FLD values, suggesting higher levels of FAW resistance. Grain yield was negatively correlated with mean FLD ($r = 0.18$, $p < 0.05$), and FCD ($r = 0.15$, $p < 0.05$). Promising maize genotypes, including CZL1310c, CML444-B, CZL15220, TL1512847 and CML491, were selected for their low mean FLD and FCD, earliness to flowering and high grain yield potential. These genotypes should be useful in developing tropical and sub-tropical maize breeding populations with partial FAW resistance and yield gains.

The third study optimised laboratory rearing and artificial inoculation of FAW onto maize plants under controlled conditions. Field-collected FAW egg masses and larvae were used to mass-produce fresh colonies of the larvae and to evaluate 63 maize genotypes for FAW resistance. The study enabled an understanding of the salient features of FAW growth and development under local environments to implement integrated FAW management strategies. Test genotypes had differential reactions to FAW infestation under controlled conditions. Several genotypes, including CML545-B, CZL1310c, VL050120, CZL16095, EBL169550, ZM4236, MM501 and Pool 16, exhibited considerable FAW resistance at the seedling and leaf-whorl growth stages, and were selected for resistance breeding. The study established a standardised laboratory and screen house-based protocol for mass rearing and artificial infestation of FAW to screen maize genotypes for resistance breeding programs in Zambia or other sub-Saharan Africa countries.

The fourth study determined the genetic diversity of 59 maize genotypes of diverse genetic backgrounds with variable resistance to FAW, using phenotypic traits and SNP-based DArT markers. The test genotypes were profiled using agro-morphological traits, FAW damage parameters, and Diversity Array Technology Sequencing-derived single nucleotide polymorphism (SNP) markers. Significant ($p < 0.001$) differences were observed among the genotypes for 13 phenotypic traits, with their phenotypic coefficient of variation ranging from 2.19 to 51.79%. Notable phenotypic variation was observed for ear position, grain yield, and

FAW-induced leaf and cob damage. The mean gene diversity and polymorphic information content were 0.29 and 0.23, respectively, reflecting a moderate level of genetic variation among the test genotypes when assessed using SNP markers. Analysis of molecular variance revealed greater genetic variance within a population than between populations. Population structure and cluster analysis grouped the test genotypes into two main clusters. Three genetically divergent, open pollinated varieties were selected for their favourable agronomic performance and FAW resistance for population improvement or hybrid breeding: Pool 16, ZM 4236 and ZM 7114. The genetic diversity detected within and among the tested populations will facilitate the breeding of maize varieties incorporating farmer-preferred agronomic traits and FAW resistance in Zambia and related agro-ecologies.

The fifth study investigated the combining ability effects and inheritance of FAW resistance and agronomic traits in maize genotypes selected for breeding. A line × tester mating design was used and 60 experimental hybrids were generated and field-evaluated in three FAW hotspot locations in Zambia. Both the general and specific combining ability effects were significant (p<0.05) for the assessed traits. Non-additive genetic effects were more important for the inheritance of grain yield and FAW-inflicted leaf and cob damage, suggesting that heterosis breeding would be the best strategy for yield gains. The narrow sense heritability (h^2) estimates for agronomic and FAW-related traits ranged from 0.14 to 0.47 and 0.37 to 0.49, respectively. The experimental hybrids CML346/EBL16469, ZM4236/CML545-B, CML346/CZL1310c, CML334/EBL173782, CML545-B/EBL169550 were among those selected with favourable specific combining ability estimates for greater grain yield, reduced days-to-50% anthesis, days-to-50% silking, FAW leaf and cob damage resistance, respectively. The selected experimental hybrids are recommended for further evaluation and breeding.

Overall, the study developed and optimized the techniques for the artificial rearing and infestation of FAW on maize under controlled conditions. Promising inbred lines and new FAW resistant experimental maize hybrids were developed involving landrace varieties and donor parents sourced from the International Maize and Wheat Improvement Center (CIMMYT). This study contributes to the development of FAW resistant maize varieties in Zambia and SSA.

DECLARATION

I, Chapwa Kasoma, declare the following;

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other University.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
a) Their words have been re-written but the general information attributed to them has been referenced.
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5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed:

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Chapwa Kasoma

As the candidate's supervisor, I agree to the submission of this dissertation

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Prof. H. Shimelis (Supervisor)

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Prof. M. D Laing (Co-supervisor)

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DEDICATION

To Anastazia L. Ziwa Kasoma, for your lively faith in me and being all that I ever hope to be.

To Francis P. Kasoma, for your legacy and final gesture to me that forever fuels my aspirations.

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ABBREVIATIONS AND ACRONYMS

ASI	Anthesis-silking-interval
BeCA	Biosciences Eastern and Central Africa
CABI	Center for Agriculture and Biosciences International
CGIAR	Consultative Group on International Agricultural Research
CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo
CV	Coefficient of variation
DAFF	Department of Agriculture Forestry and Fisheries
DArTseq	Diversity Arrays Technology sequencing
DTA	Days to anthesis
DTF	Days to flowering
ER	Ear rot
EASP	Ear aspect
EFSA	European Food Safety Authority
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuaria
EU	European Union
FAO	Food and Agricultural Organization
FAW	Fall armyworm
FCD	FAW cob damage
FLD	FAW leaf damage
FW	Fresh weight
GBS	Genotyping-by-sequencing
GCA	General combining ability
GEBV	Genomic breeding values
GMO	Genetically modified organism
GWAS	Genome-wide association study
GY	Grain yield
H^2	Broad sense heritability
HHP	Highly hazardous pesticide
HTPG	High Throughput Genotyping
ICIPE	International Center for Insect Physiology and Ecology
IGSS	Integrated Genotyping Service and Support
IITA	International Institute for Tropical Agriculture

IKS	Indigenous knowledge system
IPM	Integrated pest management
LSD	Least significant differences
MAS	Marker-assisted selection
NARES	National Agricultural Research and Extension System
NGS	Next generation sequencing
OECD	Organization for Economic Cooperation and Development
OPV	Open pollinated variety
PCA	Principal component analysis
PH	Plant height
PIP	Plant incorporated protectants
PRA	Participatory rural appraisal
QTL	Quantitative trait loci
RS	Recurrent selection
SCA	Specific combining ability
SDG	Sustainable development goals
SED	Standard error of differences
SNP	Single nucleotide polymorphism
SSA	Sub-Saharan Africa
SSR	Simple sequence repeats
SWCB	South-western corn borer
UKZN	University of KwaZulu-Natal
UNDP	United Nations Development Program
USDA	United States Department of Agriculture
USA	United States of America
ZARI	Zambia Agricultural Research Institute

INTRODUCTION TO THESIS

Background

Maize (*Zea mays* L., 2n = 2x = 20) is the world's most important commodity crop after wheat (*Triticum aestivum* L.) (FAOSTAT 2018). The grain contains essential nutrients such as carbohydrates, proteins, fats, vitamins and minerals (e.g. phosphorous, magnesium, and potassium) (Suri and Tanumihardjo, 2016). Globally, the USA, China, and Brazil are the largest producers of maize with annual production levels of 392, 257 and 82 million tonnes, respectively (FAOSTAT 2019). In sub-Saharan Africa (SSA), maize is annually produced on an estimated area of 37 million hectares (Hruska, 2019), feeding over 500 million people (Macauley and Ramadjita 2015). The average per capita annual consumption of maize in SSA ranges from 50 to 129 kg/person. Southern African people are the largest consumer of maize globally with an estimated consumption of 120 kg/person p.a. (Komher, 2018).

Despite the importance of maize in SSA, annual yields are low at 2.1 t ha^{-1}, compared to the global yield of 4.9 t ha^{-1}. This is attributable to several biotic, abiotic and socio-economic constraints that are present in the region (OECD 2018; Matova et al., 2020). There is a clear need is for high yielding cultivars with suitable quality traits, which can tolerate the various abiotic and biotic stresses, in order to sustain maize production and enhance food security in the region.

Constraints to maize production

Biotic (e.g., insect pests and diseases) and abiotic stresses (e.g., drought and heat, low soil fertility and aluminium toxicity), and socio-economic constraints (e.g., limited access to seeds of improved varieties, a lack of production inputs, high cost of fertilizer and scarcity of agricultural land) are the main impediments to maize productivity.

Among the biotic constraints, insect pests are responsible for an estimated 60% of yield losses in SSA (Mugo et al., 2018). The notable insect pests of economic importance in the region include *Busseola fusca* Fuller (Lepidoptera: Noctuidae), *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), *Cicadullina mbila* Naude (Hemiptera: Cicadellidae), *Eldana saccharina* Walker (Lepidoptera: Pyralidae), and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) (Van den Berg and Van Wyk, 2006; Assefa et al., 2010). In 2016, the invasive fall armyworm (FAW) [*Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae)] was reported in various countries in Africa. FAW has become the most devastating insect pest of economic importance

in the continent (Georgen et al., 2016; Abrahams et al., 2017), causing yield losses of 21-53% in maize (Prasanna et al., 2018).

The impact of FAW on maize and prospects for resistance breeding

More than 350 plant species are recognized hosts of the FAW. However, maize is the most preferred host. The pest is capable of causing 100% yield loss in maize under severe infestation, affecting regional supply, markets and food security (Prasanna et al., 2018). The presence of the pest in Africa has threatened the maize trade at local, regional, and international levels (FAO 2018). Public and private sectors in Africa forged research and development collaborations to mitigate the impact of FAW on maize production and the markets. Two years after the first appearance of the pest in Africa, 35 national and international organizations formed the new Fall Armyworm R4D International Consortium, led by the International Maize and Wheat Improvement Center (CIMMYT) (Kasoma et al., 2020a).

Since the appearance of FAW in SSA, various control options have been proposed, including agrochemicals, biological controls, cultural practices, landscape management and host plant resistance. Agrochemical methods are unsustainable owing to the FAW's ability to develop resistance to the active ingredients of chemical pesticides, and because most farmers in the region cannot afford to apply agrochemicals regularly. The use of classical biological control agents adapted to SSA is still being researched, aiming to identify natural enemies that can be multiplied and released. If this approach works, it will provide a stable, no-cost control option for FAW (Tefera et al., 2019). Cultural methods cannot be solely relied upon because they do not provide adequate control of the pest when used alone. Host plant resistance is the most promising option but suitable cultivars with adequate and validated resistance that are adapted to SSA climatic conditions have not yet been developed (Feldmann et al., 2019). A combination of the above control methods is known as Integrated Pest Management (IPM). IPM that incorporates host plant resistance as a core component is seen as the most sustainable management option (Prasanna et al., 2018). This requires the identification of novel sources of FAW resistance for use in resistance breeding programs. FAW resistance in maize has been developed in the regions of the world where FAW is native, including in North and South America. Recurrent and backcross selection methods can accumulate FAW resistance, which is under the control of polygenes, to develop improved cultivars (Widstrom et al., 1992). More recently, molecular tools have revealed the genetic basis of FAW resistance and have been

used for developing transgenic cultivars for FAW management in the Americas (Warburton et al., 2017).

Breeding efforts aimed at developing FAW resistant maize in Africa are in their infancy owing to the recent occurrence of the pest in the region. Development of resistant varieties for SSA will require a series of sequential steps, including the large-scale screening of diverse germplasm, identification and validation of promising genotypes, and hybridization to produce new gene combinations for evaluation under various agroecological conditions. Currently, key stakeholder institutions, including the CIMMYT and the International Institute for Tropical Agriculture (IITA), are collaborating with the National Agricultural Research Systems (NARs) in SSA on pre-breeding and breeding activities in FAW control research.

The rationale of the study

FAW is a new pest to Africa, and there are currently no African-adapted and validated FAW resistant maize cultivars available for cultivation (Prasanna et al., 2018). Consequently, the productivity of maize is substantially reduced, and many small-scale farmers' livelihoods are affected. There is a need to develop FAW resistant maize cultivars with suitable agronomic characteristics and other farmer-preferred traits to sustain maize production in the region. To this effect, pre-breeding and breeding efforts should aim at identifying promising genotypes for developing suitable FAW resistance breeding populations. There is a need for large-scale screening of African-adapted maize germplasm and crossing of promising genotypes with partial FAW resistance to develop suitable breeding populations or new generation hybrids with FAW resistance (Kasoma et al., 2020a). To enhance the adoption of FAW resistant cultivars, the resistance breeding process should also incorporate farmers' trait preferences, identified through participatory rural appraisals. Rigorous phenotyping complemented by genotyping with single nucleotide polymorphisms (SNPs) would identify suitable parental genotypes from various heterotic groups (Kasoma et al., 2020b). Further, understanding the genetic basis for inheritance of FAW resistance through combining ability analysis will enable the application of appropriate breeding methods to accelerate gains in selection.

Aim of the study

The main aim of the study was to contribute to food security in the region by breeding for host-plant resistance in maize against FAW, for incorporation in FAW-IPM programs.

Specific study objectives

The specific objectives of the study were:

i. To identify farmers' maize production constraints and preferred traits, and to assess farmers' perceived impact of FAW on maize production and productivity in Zambia;
ii. To screen maize genotypes for FAW resistance, yield, and yield-related traits by phenotyping and genotyping to select promising genotypes for crossing;
iii. To optimise the methods for laboratory rearing of FAW and to conduct artificial screening of promising maize genotypes under controlled conditions and artificial FAW infestation;
iv. To determine the level of genetic diversity for agronomic and FAW-related traits among maize genotypes using SNP markers complemented with phenotypic information to identify suitable parents for developing FAW-resistant breeding populations;
v. To determine the nature of gene action conditioning FAW resistance, yield, and yield-related traits through combining ability analysis and identify promising crosses for continued evaluation in FAW resistance breeding.

Hypotheses

The key hypotheses tested in the study were:

i. Smallholder farmers have specific trait preferences that determine the acceptability of maize cultivars, and FAW is a major constraint affecting maize production and productivity in SSA;
ii. Maize genotypes show significant variation for FAW resistance, yield, and yield-related traits under natural FAW infestation;
iii. FAW can be reared under laboratory conditions to generate sufficient pest populations for screening of maize genotypes under controlled conditions;
iv. SNP markers can discern the magnitude of genetic variation for FAW-resistance and agronomic traits among maize genotypes;
v. Selected parental lines and their resulting hybrids have significant and good combining ability effects for FAW resistance, yield, and yield-related traits.

Outline of the thesis

This thesis consists of a comprehensive review of the literature, five experimental chapters, and a concluding overview of the research (Table 0.1). The thesis follows a dominant format prescribed by the University of KwaZulu-Natal. The chapters are presented as discrete, inter-related papers and are compiled into a composite thesis following the specific objectives of the study. For this reason, there is some inevitable repetition of references and overlaps of information between chapters. The referencing was done according to the Crop Science Journal, except for Chapters 1, 2, and 4, which are already published, as indicated in Table 0.1;

Table 0.1: Outline of the thesis

Chapter	Title	Publication history
1	The fall armyworm invasion in Africa: Implication for maize production and breeding	Journal of Crop Improvement, https://doi.org/10.1080/15427528.2020.1802800
2	Farmers' perceptions of production constraints and their trait preferences in maize: implications on breeding for fall armyworm resistance in Zambia	Pending review in the Agronomy Journal- MDPI (Manuscript Id. agronomy-1019134)
3	Screening of inbred lines of tropical maize for resistance to fall armyworm, and for yield and yield-related traits	Cop Protection, https://doi.org/10.1016/j.cropro.2020.105218
4	Screening for fall armyworm resistance in maize: methodologies for controlled evaluation and genotype selection	Under review in the Journal of Applied Entomology (Manuscript ID JEN-2020-0468)
5	Revealing the genetic diversity of maize populations by phenotypic traits and DArTseq markers for variable resistance to fall armyworm	Genetic Resources and Crop Evolution https//doi.org/10.1007/s10722-020-00982-9
6	Combining ability of maize genotypes for fall armyworm resistance, yield and yield-related traits	Under review in the Journal of Crop Protection (Manuscript Id. CROPRO-D-20-01436)
-	Overview and implications of the study	

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CHAPTER 1. LITERATURE REVIEW

Abstract

Food security and livelihoods in sub-Saharan Africa (SSA) are threatened by the recent arrival of the fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith) (Lepidoptera: Noctuidae), a prolific, polyphagous insect pest of 350 host-plant species, including maize (*Zea mays* L.). In the major maize-producing countries in SSA, annual yield losses attributable to FAW are between US$ 2.5 and 6.2 billion. Presently, the FAW is an A1 quarantine pest and there is stringent cross-border control of agricultural commodities. This review presents the current impacts of FAW on sustainable maize production in SSA and the key pest management options emphasizing breeding for resistance based on best practices globally. The review analyzes suggested control strategies for FAW, based on the efforts implemented in SSA so far, and lessons learned from global regions where the FAW is already a major pest. Emphasis is placed on breeding through integrating conventional and molecular tools to improve resistance in maize and to expedite gene identification and introgression in maize for cultivar design, development and deployment. Information presented in this paper should guide sustainable management of FAW in SSA.

Keywords: breeding, crops pests, host resistance, *Spodoptera frugiperda*, sub-Saharan Africa, *Zea mays* [1]

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1.1 Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), is a highly invasive pest, which is a threat to global food production, productivity and trade. It has a wide range of hosts and therefore it attacks several plant species belonging to diverse families. FAW originated from North and South America (Goergen et al. 2016). The pest causes significant economic losses globally (Virla et al. 2008; Blanco et al. 2014; Fatoretto et al. 2017). It is highly invasive on maize, a staple crop supporting more than 500 million people in SSA (Macauley and Ramadjita 2015). In SSA, annual mean yield losses of 21-53% are reported in maize alone (Prasanna et al. 2018). An estimated annual monetary loss of US$ 2.5 to 6.2 billion is reported in SSA because of FAW infestations in the major maize-producing regions (Day et al. 2017). The Sustainable Development Goals (SDGs) set by the United Nations Development Program (UNDP) envisioned to end hunger and starvation by 2030 in sub-Saharan Africa (SSA) (Sachs 2012). However, the recent invasion and spread of FAW in Africa and Asia is likely to impede sustainable food security.

FAW affects not only food availability, but also threatens natural ecosystems, biodiversity, and local, regional and global trade (FAO 2018a). FAW, along with the native African armyworm (*Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae), and the newly identified southern armyworm (*S. eridania* (Stoll) (Lepidoptera: Noctuidae) (Bateman et al. 2018) are a threat to agriculture and ecosystems in SSA (Prasanna et al. 2018). FAW presence has been confirmed in 45 countries in Africa (CABI 2019), and currently it is considered to be one of the most invasive and dominant pests of cereal crops, including maize (*Zea mays* L.), rice (*Oryza sativa* L.), sorghum (*Sorghum bicolor* (L.) Moench) and wheat (*Triticum aestivum* L.) (FAO 2018b; Prasanna et al. 2018).

Presently, FAW is classified as an A1 quarantine pest under the European and Mediterranean Plant Protection Organization (EPPO) regulations (Abrahams et al. 2017), resulting in global trade restrictions. For instance, in January - February 2020, the European Commission intercepted plant-derived commodities, including maize, containing *S. frugiperda* from four African countries (EUROPHYT 2020). Additionally, the European Food Safety Authority (EFSA) considered including additional phytosanitary measures on sweet corn imports to minimize FAW spread into the EU zone (Jeger et al. 2018).

In Africa, the pest was first detected in 2016 (Goergen et al. 2016) and it has spread to all SSA countries, except Lesotho and Equatorial Guinea, in about two years (Assefa and Ayalew

2019). Many regions in SSA become hotspots for FAW on account of favorable climatic conditions for high levels of pest reproduction and infestation, and most popular and well-adapted cultivars of all cereal crops in SSA have succumbed to FAW infestation (FAO 2018a; Prasanna et al. 2018). This has caused both researchers and farmers to frantically search for effective control strategies, including varietal resistance against FAW in Africa. So far, farmers have used traditional methods, including hand picking and crushing of egg masses and larvae (Assefa and Ayalew 2019). There is need for modern technologies to successfully manage FAW and prevent significant yield losses in SSA (Tambo et al. 2019). In light of the above developments, the main objective of this review was to assess the present impact of FAW on sustainable maize production in SSA and the available key pest management options emphasizing breeding for host-plant resistance.

1.2 Nature and origin of the invasive fall armyworm

1.2.1 Description and biology of fall armyworm

The FAW is a polyphagous pest that attacks 350 crop species belonging to diverse families, including grasses, vegetable crops and shrubs (Balla et al. 2019). It belongs to the genus *Spodoptera* Gueneé (Lepidoptera: Noctuidae), consisting of 31 species, including the African armyworm (*S. exempta* Walker), the cotton leaf worm (*S. littoralis* Boisduval), the beet armyworm (*S. exigua* Hübner) (Abrahams et al. 2017) and the newly identified southern armyworm (*S. eridania* Stoll) from West Africa (Bateman et al. 2018). FAW attacks in Nigeria were initially attributed to indigenous species *Spodoptera guenée* (Lepidoptera: Noctuidae), *S. exigua* (beet armyworm) and *S. exempta* (African armyworm) (Goergen et al. 2016). Similarly, the pest was erroneously labeled as the common corn borer, the African armyworm or cotton leaf worm in southern Africa. Its aggressive feeding behavior and high fecundity distinguish FAW from other armyworms and Lepidopteran pests (Goergen et al. 2016).

Under favorable conditions, the female FAW lays 1500-2000 eggs during its life cycle. The eggs hatch into neonate larvae within four days (Simmons and Lynch 1990; Prasanna et al. 2018). The larval stage consists of six instars, followed by a pre-pupa stage, during which the larva falls and burrows into the ground to a depth of 7.62 to 10.16 cm for 2 to 4 days prior to pupation (Hardke, Lorenz, and Leonard 2015). Pupation may last from 7 to 14 days, depending on the soil temperature. Emerging insects from the pupal stage move to the soil surface, becoming adults and infesting growing plants. The entire life cycle of an individual FAW may take up to four weeks, whereas a generation consisting of individual larvae emerging from egg

masses of similar age can last from 80 to 90 days (Abrahams et al. 2017). FAW's entire life cycle, reproduction and distribution depends on temperature conditions, crop season, presence of host-plant species and fecundity. The fast spread of the pest in SSA is probably linked to the moth's notable dispersal capacity, prevailing wind conditions and availability of varied host species. FAW moths are nocturnal and are capable of flying for 100 km in one night (Zhou et al. 2020). Jeger et al. (2018) reported a long-distance flight by other FAW-related noctuid pests, including *S. exigua*, from North Africa, reaching the UK and Spain. FAW prefers grass hosts, such as maize, sorghum and Bermuda grass. Older larvae exhibit a nocturnal and voracious feeding that peaks during the last two instar stages (Harrison et al. 2019).

1.2.2 Biotypes of fall armyworm and genetic differentiation

There are two known biotypes of FAW, namely the corn (C) and rice (R) biotypes (Abrahams et al. 2017). The two biotypes were distinguished through the use of single nucleotide polymorphism (SNP) markers (Sibanda 2017). Morphologically, the biotypes are identical (Nagoshi et al. 2018), but their developmental stages differ in growth rate, pupal weight and oviposition period (Pashely et al. 1988). Furthermore, the two biotypes were believed to be sexually incompatible (Pashely et al. 1988). However, successful mating of the R-biotype females with the C-biotype males has been reported, although reciprocal crosses failed (Pashely et al. 1988). Mating compatibilities between the two biotypes may result in new and aggressive variants. The two biotypes are genetically and behaviorally different, and hence they need different control methods. Kuate et al. (2019) reported differential response of the FAW biotypes to crop protection chemicals, with different mortality rates.

Nagoshi et al. (2018) reported the existence of an FAW variant of the R-biotype. This variant is reportedly unique to Africa and has been detected in Togo, Kenya and the Democratic Republic of Congo. Haplotype comparison using 300 FAW samples from across six countries in Africa, based on SNPs analysis, enabled preliminary establishment of the invasion history and migratory patterns of the FAW biotypes and their variants (Nagoshi et al. 2018). There is need for National Agricultural Research and Extension Systems (NARES) to collaborate on the collection of representative samples, including from southern Africa, to enable validation and confirmation of the above findings.

Morphological characterization and SNPs analyses using the mitochondrial cytochrome oxidase subunit (COI), which encodes for a functional gene, were the dominant forms of FAW biotype differentiation in Africa (Goergen et al. 2016; Nagoshi et al. 2018). However, there is

a suspected disruption of the mitochondria-based marker, and this may limit proper biotype identification and hence subsequent management strategies (Ingber, Mason, and Flexner 2017). As FAW invades more countries in Africa, it is being closely monitored for further biotype development (Nagoshi et al. 2018). NARES, in collaboration with the private sector and international research organizations, can employ complementary methods to reliably differentiate FAW biotypes. This will enable the designing of targeted and sustainable integrated pest management (IPM) strategies against FAW. Advances in the application of molecular markers are widely reported, which may provide a foundation for continued application of the latest molecular tools in FAW resistance breeding in Africa (Table 1.1)

Table 1.1. Molecular markers used in FAW research in cereal crops and possible applications in SSA

Marker	Application	Crop	Country	Possible application in SSA	Reference
RFLP†	Distinguishing FAW from other noctuid pests	Maize (*Zea mays* L.)	USA	To distinguishing several *Spodoptera* species with which the FAW has been mistaken	Lewter et al. (2006)
AFLP	Comparison of intra and inter strain genetic variability	Maize	USA	For prescribing appropriate crop protection chemicals for use in IPM	Clark et al. (2007)
AFLP and SSR	Linkage mapping of FAW resistance genes	Buffel grass (*Cenchrus ciliaris* L.), Bermuda grass (*Cynodon dactylon* L.), Zoysiagrass (*Zoysia sp.)*	USA	For synteny studies involving related grass species and to providing a foundation for identifying FAW resistance genes in maize and other crops	Jessup et al. (2006; 2011)
SSR	First developed for discriminating FAW populations		USA	For tracing the origins of FAW populations	Arias et al. (2010)
SSR	Ascertain FAW identity and detect candidate FAW migrants	Maize	Brazil	To distinguishing among *Spodoptera* species and tracing the origins of FAW populations	Pavinato et al. (2013)
SNP	Mapping of insect resistance QTL	Maize		To use in marker-assisted selection in FAW resistance breeding after QTL validation	Brooks et al. (2007)
SNP	FAW population structure based on host-related factors and genetic distinctiveness			For tracing of FAW population origin and determining appropriate crop protection chemicals for IPM	Silva-Brandao et al. (2018)
SNP	Validation of the origin of African FAW population	Maize and sorghum (*Sorghum bicolor* [L.] Moench)	Sao Tome and Principe, Burundi, D.R. Congo, Tanzania, Kenya	For determining appropriate crop protection chemicals	Nagoshi et al. (2018)
SNP	Identification of FAW strains present in Africa	Maize and sorghum	Zambia, Ghana Chad, D.R. Congo, Togo, Tanzania, Kenya South Africa	For determining appropriate crop protection chemicals	Nagoshi et al. (2019)
SNP	Determining the most possible routes of FAW entry into Africa	Maize	Ghana	For strengthening preparedness, monitoring and surveillance	Cock et al. (2017)
SNP	Identification of major QTL for FAW resistance	Maize	USA	Foundation for incorporating in marker-assisted selection in FAW resistance breeding after QTL validation	Womack et al. (2020)

†(RFLP) Restriction Fragment Length Polymorphism; ‡ (AFLP) Amplified Fragment Length Polymorphism; § (SSR) Simple Sequence Repeat; (SNP) Single Nucleotide Polymorphism

In FAW genetics research, molecular markers can be used to assess the effectiveness of *Bt* (*Bacillus thuringiensis*) technology for countries adopting it for FAW control. The development of FAW biotypes resistant to the *Bt* gene *Cry1F* is associated with a mutation in an ATP-binding cassette sub-family that functions as a *Cry1F*a receptor in susceptible insects (Banerjee et al. 2017). There is need for validation of molecular markers that can reliably detect mutations in this ATP-binding cassette region to provide a means of predicting the effectiveness of *Bt* technologies in a region commonly infested with a given strain of FAW. This information would contribute to the discussion on whether the adoption of transgenic crops in SSA could adequately reduce FAW-related crop losses. Application of the Next Generation Sequencing (NGS) technologies, such as genomic selection, is key for FAW resistance breeding in SSA. Recently, the following genotyping platforms were established in SSA in Kenya: The Integrated Genotyping Service and Support (IGSS) and the High Throughput Genotyping (HTPG), which are hosted by the Biosciences Eastern and Central Africa (BeCA) and Excellence in Breeding (EiB), in that order. The two platforms are making the NGS technologies more accessible and affordable for several breeding programs in the region (Dreisigacker 2016). Kakumani et al. (2014), Banerjee et al. (2017) and Warburton et al. (201x7) have successfully utilized these tools to identify FAW resistance genes in maize for effective breeding.

1.2.3 Origin and spread of the fall armyworm

FAW originated from the North and South America (Goergen et al. 2016). Mexico, Argentina, Puerto Ricco, Brazil, Florida, and Canada are among the hotspot areas traditionally associated with the recurring presence of FAW since 1928 (Davis, Williams, and Wiseman 1989). In Africa, the first FAW occurrence was detected and reported in West Africa, including in Nigeria, São Tomé e Príncipe, Togo and Benin in 2016 (Goergen et al. 2016). The accurate time, port and modes of entry of FAW into Africa are debatable. Could FAW have occurred in SSA prior to 2016 and then built sufficient populations before reaching epidemic proportions in the region? This is probably unlikely because the FAW, unlike the African armyworm, does not require a threshold population to initiate feeding in a maize crop (Assefa and Ayalew 2019). Thus, if the pest had occurred before 2016, FAW-like damage should have been reported by farmers or researchers in Africa. Yield loss in maize attributable to stalk borer and the African armyworm was rather widely reported in SSA. Since 2011, *S. littoralis* was the most common *Spodoptera* species detected from SSA in living plants of the following genera: *Rosa*, *Capsicum* and *Solidago*. In June 2017, the first commodity containing FAW was recorded from

SSA when *S. frugiperda* was detected on *Rosa sp.* readied for export from Zambia (EUROPHYT 2020). In the 2016/2017 crop season, the FAW spread into southern and East African countries and caused severe crop losses (Stockad 2017). Plant-based commodities, baggage or cargo are among the means by which FAW is suspected to have spread between countries in Africa and globally (EUROPHYT 2020). This warrants a coordinated system in tracking commodity transfers in Africa and elsewhere for effective monitoring and control of the FAW. In April 2017, at least 16 African countries had confirmed the presence of FAW, and in October 2017, more than 30 African countries had confirmed the incidence of FAW (Abrahams et al. 2017; FAO 2018a). By December 2018, 41 out of 54 African countries had confirmed the presence of FAW, whereas three countries, namely, Gabon, Equatorial Guinea and Republic of Congo, suspected its presence, pending confirmation (FAO 2018b). Lesotho is the only country in Southern Africa without FAW presence (CABI 2019). In designing integrated FAW management strategies for SSA, a detailed investigation on the various factors that may contribute to hindering FAW infestation and distribution in Lesotho is crucial. This information may also be useful for the EU, which launched a study of FAW-free areas in Africa for possible production of specific niche market for agricultural commodities (Jeger et al. 2018). In mid-2019, four more countries confirmed the presence of FAW, bringing the total number of the affected countries in Africa to 45 (CABI 2019) (Figure 1.1). Apart from the countries in mainland Africa, FAW presence is also confirmed in the islands associated with Africa, such as Madagascar. In mid-2018, FAW spread beyond Africa to Asia, where it was first detected in the Chikkaballapur area of the Karnataka State of India (CABI 2018). It has since been detected in more than 10 states in India. The latest reports documented the confirmed presence of FAW in eight other countries in Asia, viz., China, Indonesia, Malaysia, Sri Lanka, Bangladesh, Myanmar, Vietnam and Thailand (Balla et al. 2019).

Figure 1.1. Map of Africa showing spread of FAW. Presently, 45 countries (green colour) have confirmed FAW presence, Equatorial Guinea has suspected presence and the six countries in yellow, including Mauritania, Morocco, Tunisia, Algeria, Libya and Lesotho have not reported pest presence.

1.3 Factors contributing to the distribution and invasiveness of FAW

The key factors for FAW's distribution and crop damage are favourable environmental conditions that are all present in Africa, a diverse host range, a high rate of reproduction, rapid nocturnal flight and a gregarious feeding habit. Additional factors include a high intra- and inter-specific competitive ability, a lack of accurate forecasting, monitoring and FAW-management options in most SSA countries (Graham 2018).

1.3.1 Environmental factors

Warm temperatures, high humidity and drought conditions accelerate FAW infestation (Goergen et al. 2016; Stockad 2017). These conditions enhance FAW's growth, development and ecological succession. High temperature conditions (16 to 30°C) enhance the rapid initiation and completion of its instar stages, leading to sporadic invasions. Early et al. (2018)

reported that most agro-ecological and environmental conditions in SSA support the year-round presence of FAW. Further, Prasanna et al. (2018) noted that warmer temperatures increased the rate of egg hatching, larval and pupal development, resulting in a short life cycle.

1.3.2 Host ranges

Despite the wide host range of FAW, its two biotypes mainly prefer maize and rice (Prasanna et al. 2018). Casmuz et al. (2017) reported that FAW caused variable damage on the following families of plants in decreasing proportions: Poaceae (35.5%), Fabaceae (11.3%), Solanaceae and Asteraceae (4.3%), Rosaceae and Chenopodiaceae (3.7%), and Brassicaceae and Cyperaceae (3.2%). Therefore, the family Poaceae, which contains the major cereal crops, including maize, serves as the major primary host of FAW. During the larval stage, the FAW acquires the "armyworm habit", and it often spreads in large numbers, aggressively defoliating its host plants (Abrahams et al. 2017). FAW's primary and alternate hosts, including cereals, like wheat, and grass species growing during the offseason periods, extend its survival opportunities, serving as green bridges between plant species and cropping periods (Prasanna et al. 2018). Monocropping systems practiced by many farmers in SSA exacerbate the problem of FAW (Midega et al. 2018).

1.3.3 Nature of reproduction and feeding habit

The female FAW oviposits on susceptible host plants, resulting in larvae colonizing the host (Rojas, Kolomiets, and Bernal 2018). Non-selective oviposition can occur on alternate hosts when the FAW population is large, although colonization might be unsuccessful. Feeding intensity increases during FAW's transition from early to late instars. The first instar larvae cause elongated lesions that resemble "clear window-panes" on the plant's leaves, while the sixth instar larvae tear and tatter huge portions of the leaves, resulting in more severe damage. On maize plants, the pest feeds and deposits its eggs on almost every plant part, accelerating its establishment and increasing its population (Goergen et al. 2016).

1.3.4 Intra-and inter-specific competition ability

FAW has strong inter- and intra-specific competition ability, including through cannibalism (Goergen et al. 2016). FAW will feed on maize stem borers, stopping other borers from feeding inside leaf whorls. Further, older FAW larvae may cannibalize younger FAW larvae within the

leaf whorls. This form of cannibalism confers both direct and indirect benefits on FAW. Direct benefits include increased survival, development and fecundity, whereas indirect benefits occur by way of removal of potential inter- and intra-specific competitors (Kuate et al. 2019).

1.3.5 Lack of accurate forecasts, monitoring and pest management

The FAW is a new pest species in Africa. In SSA, there is limited knowledge about the pest *per se*, and especially about its biology and ecological adaptation to the varied environmental conditions. Weather-dependent forecasts are key for exploiting information technology (IT) tools, such as remote sensing, because weather affects FAW occurrence and the level of damage it causes (Balla et al. 2019). Unreliable data on weather, agro-ecological conditions, cropping systems and socio-economic conditions in SSA prevent national protection services from establishing a good early-warning system. There is need to understand the migratory nature of the pest, and to develop surveillance capacity across Africa for the effective monitoring of FAW, to be able to deploy effective management strategies (FAO 2018b).

Viable approaches for generating accurate forecasts and effective timely treatments include the use of pheromone traps (FAO 2018a). Pheromone traps assist in determining the economic threshold level of the pest population in a given area and guide on phytosanitary-treatment decisions (Prasanna et al. 2018). Scouting has also been described as a useful tool for FAW monitoring (Prasanna et al. 2018). The presence of FAW eggs on more than 5% of the crop stand, and damage symptoms on more than 25% of the stand, indicate the need for the application of pesticide sprays (Sibanda 2017). Further, a threshold of >5% excised leaves of a young seedling plant population is an additional indicator of need for initiating FAW control treatment (Abrahams et al. 2017). Presently, some SSA countries are actively undertaking farmer training, field monitoring, information and resource sharing for more accurate pest identification and management (FAO 2018a). This contributes to the organized control of the pest regionally. Mobile phone application programs, such as FAMEWS, are increasingly being used for early warning and rapid monitoring and management of FAW (http://www.fao.org/e-agriculture/news/fao-develops-mobile-phone-app-monitor-fall-armyworm-faw-africa)

1.4 Economic importance of FAW in SSA

1.4.1 Impact of FAW on maize production and productivity in SSA

1.4.1.1 Overview of maize production trends and constraints

Maize is an important cereal grown on approximately 37 million hectares in SSA (Hruska 2019). The mean yield of maize in SSA is 2.1 t ha^{-1}, compared with the global mean of 4.9 t ha^{-1} (OECD 2018). Low yields in SSA are attributed to abiotic stresses (e.g., recurrent drought, heat, flooding, and poor soil health, including aluminium toxicity), biotic stresses (e.g., pests, diseases, *Striga*) and socio-economic constraints (e.g., a lack of access to seed of improved cultivars and other inputs, such as fertilizers). Notable maize diseases in SSA include maize streak virus, grey leaf spot (*Cercospora zeae-maydis* Tehon & Daniels and *C. zeina* Crous & Braun), northern corn leaf blight (*Setosphaeria turcica* Leonard & Suggs), common rust (*Puccinia sorghi* Schwein.) and the maize lethal necrosis (MLN). MLN is reported predominantly in East Africa and is caused by the sugarcane mosaic virus and maize chlorotic mottle virus (Boddupalli et al. 2020). Also, maize is attacked by various insect pests that cause 60% yield loss in Africa (Mugo et al. 2018). The most challenging field insect pests are the maize leaf hopper *Cicadulina mbila* Naude (Hemiptera Cicadullidae), a potential vector of the maize streak virus, the maize stalk borer *Busseola fusca* Fuller (Lepidoptera: Noctuidae), and *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), which predominantly occur in East and southern Africa. In West and Central Africa, the African sugarcane stalk borer (*Eldana saccharina* Walker [Lepidoptera: Pyralidae]) and the African pink borer (*Sesamia calamistis* Hampson [Lepidoptera: Noctuidae]) are among the economically important pests of maize (Van den Berg and Van Wyk 2006; Assefa et al. 2010). Other insect pests, such as *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) and weevils (*Sitophilus granaries* L. [Coleoptera: Dryophthoridae], *S. zeamais* Motschulsky and *S. oryzae* L.) inflict severe damage on stored maize grain (FAO 2018a; Mugo et al. 2018). The African armyworm (*S. exempta*) and the southern armyworm (*S. eridania*) are among the species of economic importance in maize. The recent expansion and distribution of FAW to SSA have had a devastating effect on food security and livelihoods (FAO 2018b; Prasanna et al. 2018).

The major socio-economic constraints affecting maize production under the predominantly smallholder-farming systems in SSA include limited access to improved seed, fertilizers, herbicides, pesticides, poor access to credit opportunities for purchasing agricultural inputs and inadequate modern grain storage facilities (FAO 2018a). Successful and sustainable maize

production for food security and trade depend on strategies to minimize the above constraints in SSA.

1.4.2 Damage caused by FAW in maize

FAW attacks all above-ground parts of maize (leaves, ears and tassels), often leading to severe defoliation and reduced photosynthesis (Abrahams et al. 2017). FAW damage can cause up to 100% crop failure. The crop growth stage during infestation has a marked relationship with yield loss (Assefa and Ayalew 2019; Baudron et al. 2019). For instance, in Ethiopia, a yield loss of 30% was reported when infestation occurred during the late-whorl growth stage, corresponding to Stage 1.5 of maize growth (Hanway 1966; Assefa and Ayalew 2019). The FAW larva and nature of damage on maize are presented in Figure 1.2. Different growth stages of maize and the impact of FAW infestation and resulting damage are presented in Table 1.2.

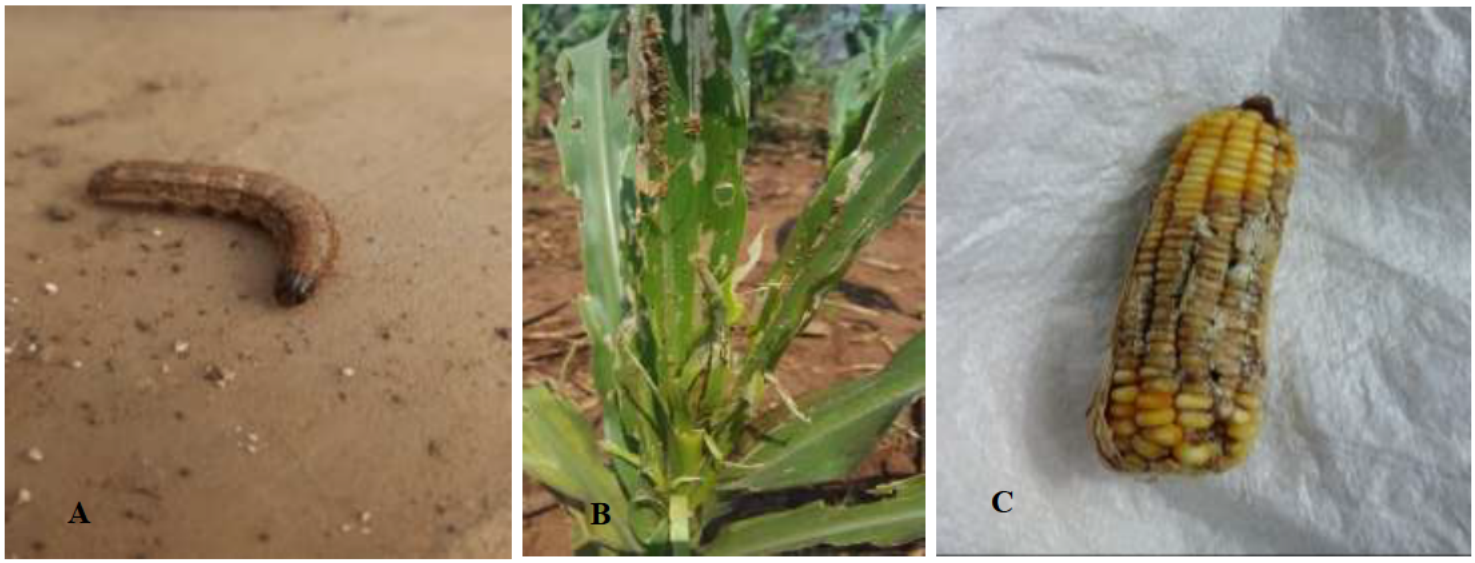

Figure 1.2. The FAW larva (A) and the nature of foliar (B) and cob (C) damages on maize.

Table 1.2. Growth stages of maize† and description of Fall armyworm infestation and damage

Growth stage (V/R codes‡ and description)	FAW damage description	References
V1-V10: Emergence and early- to mid- whorl stage Crop establishment and stalk elongation	• Leaves damaged • Pinholes or small elongated lesions on leaves caused by neonate larval feeding • Small and large leaf portions consumed by late instar FAW larvae	Williams et al. (1998); Mueller and Sisson (2013)
V11-V12: Late whorl stage Tassel begins to develop inside leaf whorl	• Leaves, tassels and ears are damaged • Reduced photosynthesis, poor ear and tassel development and yield loss	Williams et al. (1998); Mueller and Sisson (2013)
V13-V14 Tassel emerges from leaf whorl	• FAW larvae moved to tassel and feed on the anthers • Tassel appears chopped up at the top end • Reduced pollen production	Williams et al. (1998); Mueller and Sisson (2013)
R1 Start of ear formation closest to the top leaf	• FAW larvae moved to base of a developing ear and feed on emerging silks	Williams et al. (1998); Mueller and Sisson (2013)

† (Mueller and Sisson 2013)

‡ (V/R Codes) Vegetative and reproductive maize growth stages; V1-V14: Maize vegetative stages 1-14; R1- Maize reproductive stage 1

Infestation occurring at the seedling and early-whorl stages (4 to 8 leaf stage) causes defoliation and apical meristematic damage. Typically, defoliation of maize caused by FAW rarely exceeds 50% because of the ability of maize to compensate for foliar damage (Chimweta et al. 2019; Hruska 2019). However, low-yielding varieties, poor soil nutrition and field-management practices affect the ability of maize to compensate for foliar damage caused by the pest (Hruska 2019). During the mid-whorl stage (5 to 8 leaf stage), the fourth to sixth instar larvae bore into the whorl, causing extensive damage to the plant. This results in stunted or deformed plants, plant death and reduced plant population, leading to significant yield loss or crop failure. At the tasseling stage (12 to 14 leaf stage), the FAW larvae move toward the tassel, causing injury and reduced pollen production and fertility. At the post-tasseling stage (the first reproductive stage when the ear begins to form), the larvae move to the developing ear, feeding on the silks, leading to reduced fertilization and hence a reduced number of kernels per ear.

1.4.3 FAW associated yield losses and economic implications of damage on maize

In SSA, there is limited empirical data on yield loss assessment. Yield losses associated with FAW damage in maize were reported based on survey data obtained on farmers' perceptions in West, East and southern Africa. Predicted yield losses in maize, attributable to FAW, were 22 to 67% in West Africa (Day et al. 2017), 32% in East Africa (Kumela et al. 2018) and 21 to 53% in Southern Africa (Prasanna et al. 2018). Subsequent assessments based on FAW scouting in selected farmers' fields reported an FAW-related yield loss of 12% (Baudron et al. 2019). Nevertheless, previous assessments heavily relied on farmers' perceptions, or were based on inadequate sampling of FAW-infested fields. In Asia, yield losses attributable to FAW were estimated at 33%, closer to those from SSA (Balla et al. 2019).

Maize is a key food security and economic crop, especially in the southern African region, where it contributes up to 30% of the total caloric intake of the population. The per capita annual consumption of maize in Zimbabwe, Zambia, and Malawi is 153, 168 and 181 kg, respectively (Aquino et al. 2001). Grant, Woolfaardt, and Louw (2012) reported a daily mean consumption of 252.7g/person of maize in South Africa, Zambia and Malawi, making it the most important staple crop in the region. The per capita annual consumption is 100 kg in Kenya, 47 kg in Ethiopia (Onono, Wawire, and Ombuki 2013; Yami, Meyer, and Hasan 2020), and 17 kg in Nigeria (Girei et al. 2018). Therefore, reduction in maize yield attributable to FAW contributes to direct losses in household incomes and has a negative impact on the national gross domestic product through reduced regional trade and market access (Otipa et al. 2017). Total maize production areas in selected African countries, proportionally affected by the FAW invasion during the first three months after its detection in southern Africa, are shown in Figure 1.3. Zimbabwe, followed by Zambia, were among the countries most severely affected by FAW (http://www.fao.org/africa/news/detail-news/en/c/471000/; Stockad 2017). In SSA, 13.5 million tons (>20% of total regional production) of maize, valued at USD$ 3058.8 million, is being lost annually because of FAW damage (Abrahams et al. 2017).

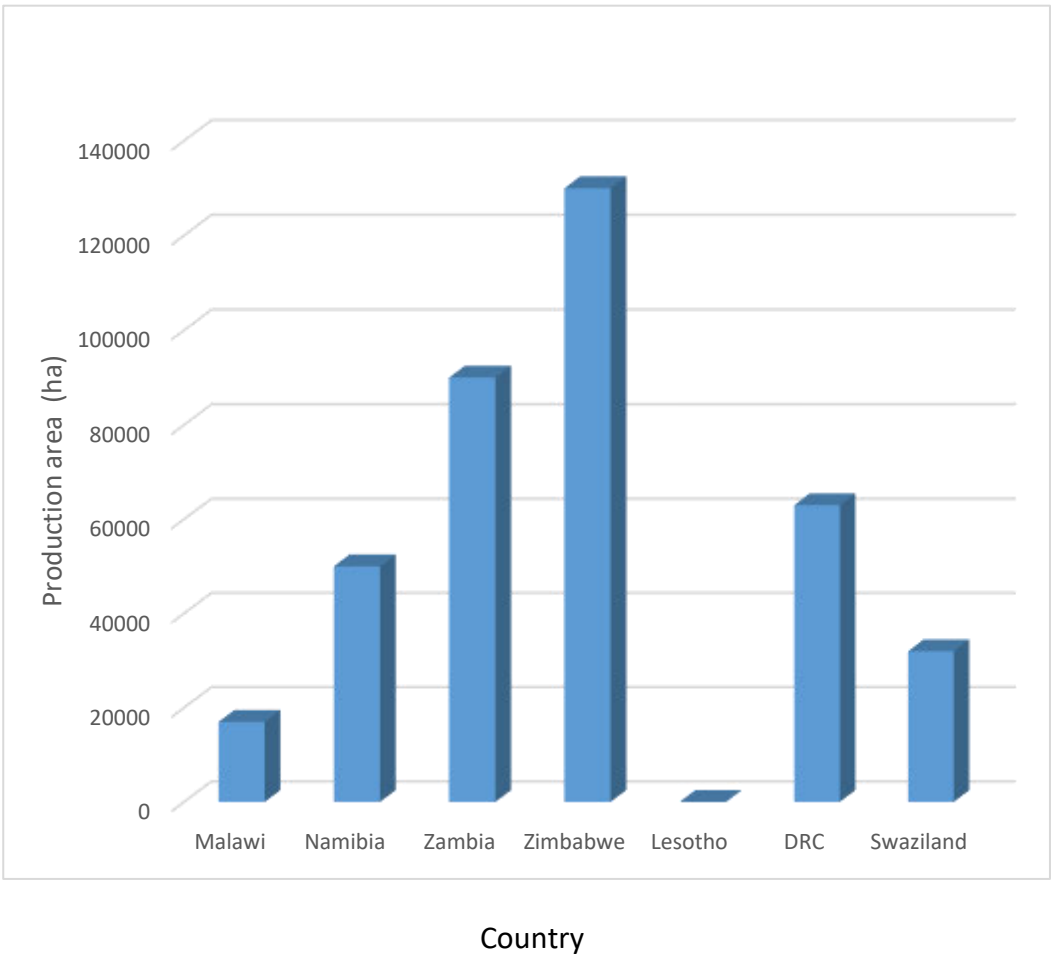

Figure 1.3 Total maize production area affected by the fall armyworm in selected African countries during the first three months of its occurrence in the 2016/2017 cropping season (Stockad, 2017; http://www.fao.org/africa/news/detail-news/en/c/471000/)

1.5 Efforts to mitigate FAW impacts in SSA

Since the arrival of FAW in Africa, information gathering on useful mitigation strategies is ongoing from within and outside the continent. National and regional task forces were established to facilitate knowledge sharing for concerted research efforts and progress tracking on the FAW status and its control in SSA. FAW working groups have been established in East, West and Southern Africa, enabling partner countries to foster joint efforts toward managing FAW (CGIAR 2018).

1.5.1 Control strategies

Several control strategies have been recommended, which can be tested and adopted in SSA. These strategies include cultural practices, chemical control (Abrahams et al. 2017); biocontrol agents; crop plant incorporated protectants, host-plant resistance and integrated FAW

management (Prasanna et al. 2018). Recommended cultural practices include timely planting following the main rainfalls, crop rotation, preferably with non-grass species, such as soybean, burning of crop residues, and landscape management by clearing major and alternate hosts around maize fields (Abrahams et al. 2017). Cultural practices are the starting point to minimize pest populations and can be considered preventative measures. Presently in SSA, application of chemical pesticides is reportedly the most commonly used control strategy for FAW (Stockad 2017). Biocontrol agents, such as entomopathogenic fungi (e.g., *Metarhizium anisopliae* (Metschn.) and nucleopolyhedroviruses (e.g., the *Spodoptera frugiperda* nucleopolyhedrovirus) are known to be natural enemies of FAW in the Americas. Abrahams et al. (2017) reported a 70% success rate in controlling FAW by using parasitic wasps in Brazil. Successful deployment of biocontrol agents for use in SSA requires targeted research on the interactions between FAW biotypes and their natural enemies. Transgenic crops provide an option for FAW control and are the main form of control used against the pest in America (Hruska 2019). Host-plant resistance based on the plant's intrinsic ability to resist or tolerate FAW herbivory, is an economic and environmentally friendly approach that is useful to both smallholder and commercial farmers. It relies on the various defense mechanisms of plants against insect pests. These mechanisms include plant insecticidal biochemicals (protease inhibitors, such as Mir1-CP and maysin) (Lyons, Manners, and Kazan 2013), physical defenses (trichomes, thorns, cuticles, lignin and silicon phytoliths) (Lourenco et al. 2017), and physio-chemical defenses (jasmonic acid, salicylic acid and ethylene) (Lyons, Manners, and Kazan 2013). These defense systems are important to consider when selecting parents for host-plant resistance breeding. Host-plant resistance mechanism comprises three categories: non-preference (antixenosis), antibiosis and tolerance. Non-preference occurs when the host plant has a repellent effect on the pest. Antibiosis causes adverse effects on the insect pest's growth and development, e.g., reduced oviposition and feeding or reduced insect survival. Pest tolerance is exhibited by particular host plant cultivars that have the ability to compensate (or recover from) damage caused by insect populations that would cause greater damage to other cultivars of the same species under given environmental conditions (Acquaah 2012; Togola et al. 2017). Integrated FAW management involves two or more management strategies that may provide synergistic effects on pest control before significant damage is inflicted (FAO 2018a). International research organizations, such as the International Maize and Wheat Improvement Center (CIMMYT; Centro Internacional de Mejoramiento de Maíz y Trigo), International Institute of Tropical Agriculture (IITA), Center for Agriculture and Biosciences International

(CABI), International Center for Insect Physiology and Ecology (ICIPE), United Nations' Food and Agriculture Organization (FAO), national research institutes, such as Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) in Brazil and the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), and the private sector, are actively working to design cost-effective FAW control strategies. Their activities have encompassed host-resistance breeding, testing of biocontrol agents, and training of farmers on early detection of FAW and the development of practical control measures. CIMMYT and IITA are aiming to introgress novel FAW resistance genes into susceptible varieties to develop cultivars that can be released in SSA.

1.5.2 Application, progress and recommendations of proposed control strategies

1.5.2.1 Cultural and landscape management control

In pursuit of food security, and specifically to control FAW, smallholder farmers in SSA use various cultural practices. In Zambia, for instance, resource-poor farmers have no access to chemical pesticides; they instead use a mixture of sawdust and ash or lime (CGIAR 2018). This product is applied directly into the whorl as an alternative control technique, which can be regarded as one of the indigenous knowledge systems (IKS). This remedy, however, is not considered efficacious (Abrahams et al. 2017). Similarly, farmers in some parts of Africa, such as Malawi, have resorted to using fish soup mixed with sugar solution, a bait to attract natural enemies of the FAW, in an attempt to reduce pest populations in their fields (Harrison et al. 2019). The use of sugar solution for FAW control was reported by Canas and O'Neil (1998), who indicated that, with its use, both FAW populations and leaf damage in maize were significantly reduced. In some instances, farmers pluck and remove FAW larvae by hand, or apply fine sand into the leaf whorl to abrade, and subsequently kill, the larvae as they move. In addition, landscape-management options, such as the push-pull technique, wherein a pest-repellent crop "pushes" the pest toward a pest-attractant that "pulls" the pest, can be used to control FAW. *Desmodium intortum* (Mill.) Urb. is an example of a pest-repellent plant that has been used effectively as an intercrop with maize, whereas *Brachiaria* cv. Mulato II was used as an attractant border crop to minimize FAW populations in maize in East Africa (Midega et al. 2018). Napier grass (*Pennisetum purpureum* Schumach) was also investigated as a potentially suitable pest attractant in a maize-legume intercrop system, aiming for pest containment, and targeted use of suitable control strategies (Khan et al. 2018). However, cultural practices alone are unlikely to provide adequate FAW control (Abrahams et al. 2017).

1.5.2.2 Chemical control

Synthetic chemicals are widely used, with varying success rates, to control FAW globally. Methyl parathion, chlorpyrifos, methamidophos, and phoxim are among the common chemical pesticides used in Mexico (Sisay et al. 2019). In the USA, Radiant, Orthene, and Larvin reportedly achieved 60% FAW mortality (Belay, Huckaba, and Foster 2012). Because of the unexpected appearance of the FAW in SSA, there were no registered chemical pesticides for use in SSA; thus, emergency registration was undertaken in some countries. In southern Africa, for instance, in 2017, some chemical pesticides were subsequently given emergency registration, including Devacarb [active ingredient indoxacarb, DVA Chemicals (Pty) Ltd], Delegate, Radiant and Uphold (active ingredient spinetoram, Corteva Agriscience) (CropLife 2017). In SSA, South Africa was among the first countries to grant emergency registration of a number of pesticides for the control of FAW (Table 1.3). A number of pesticides were used in SSA, such as methomyl (Lannate LV), methyl parathion (Methyl 4EC), lindane (Germate Plus), chlorantraniliprole (Coragen 200 SC), spinetoram (Radiant 120 SC), carbaryl (Sevin XLR Plus), dimethoate 40% (Agro-Thoate 40% EC), spinosad (Tracer 480 SC), lambda-cyhalothrin (Karate 5 EC), Malathion 50% EC (Malathion), chlorantraniliprole + lambda-cyhalothrin (Ampligo 150 SC), and Imidacloprid + Betacyflutherine (Thunder 145 OD O-TEQ). These provided variable efficacy and success rate in the region (Bateman et al. 2018; Sisay et al. 2019). Results based on a preliminary insecticide screening indicated that Radiant, Tracer, Karate, and Ampligo were most effective, whereas Malathion 50% EC and Carbaryl were relatively less effective against FAW (Sisay et al. 2019). In Ethiopia and Kenya, 48% of the farmers reportedly used chemical pesticides against FAW in 2018 (Kumela et al. 2018). The use of unregistered synthetic chemicals has been reported in SSA because of the lengthy procedures involved in registering chemical pesticides (CGIAR 2018; Sisay et al. 2019). To coordinate efforts for successful FAW management, six East African countries, viz., Kenya, Rwanda, Burundi, Uganda, Tanzania and South Sudan, piloted harmonized pesticide efficacy guidelines. The guidelines were aimed at accelerating joint testing and registration of pesticides for FAW control in the region (CGIAR 2018). The most recent crop protection chemicals widely used to control FAW in southern Africa include Fortenza Duo and Lumivia developed by Syngenta and Corteva Agriscience, respectively. These products are both seed treatment formulations that are designed to control FAW attack during the first 30 days of crop establishment after sowing (Alyousuf et al. 2018; Triboni et al. 2019). Further studies are on track to establish the efficacy of the chemicals against FAW in other SSA countries.

Table 1.3. List of agrochemicals first registered to control Fall armyworm in South Africa†

Active ingredients	Brand name	Insecticide mode of action	Resistance group	Crops
Benfuracarb / Fenvalerate	Oncol Super 220 EC	Acetylcholinesterase (AChE) inhibitor, systemic and contact	1A and 3	maize, sweetcorn, and sorghum
Beta-cypermethrin	Akito	Sodium channel modulator, contact	3A	Cruciferae, maize, sorghum, sweetcorn, wheat, tomato, pea, lupin, lucerne, groundnut
Carbosulfan	Marshal 48 EC	Acetylcholinesterase (AChE) inhibitor systemic	1A	Maize
Cartap hydrochloride	Ag-Tap 500 SP	Nicotinic acetylcholine receptor (nAChR) channel blocker, systemic and contact	4C	barley, cabbage, canola, maize, onion, potato, sorghum, soybean, sugarcane, sunflower, sweetcorn, wheat
Chlorantraniliprole	Coragen, Prevathon	Ryanodine receptor modulator, contact	28	cotton, maize, sorghum, sweetcorn, sugarcane, potato
Chlorantraniliprole / Lambda-cyhalothrin	Ampligo	Ryanodine receptor and Sodium channel modulator, contact	3 and 28	barley, canola, maize, sweetcorn, groundnuts, soy bean, sunflower, wheat
Chlorpyrifos	Avi Klorpirifos, Agropyrifos, Pyrinex 480 EC, Cropchem Chlorpyriphos	Acetylcholinesterase (AChE) inhibitor, contact	1B	maize, pastures, potato
Chlorpyrifos / Cypermethrin	Cyperfos 500 EC	Acetylcholinesterase (AChE) inhibitors and Sodium channel modulator and Contact	1B and 3B	maize, wheat, sorghum
Diflubenzuron	Dimilin 25 WP, Dimilin SC 48	Inhibitors of chitin biosynthesis, contact	15A	maize, sweetcorn, potato
Emamectin benzoate	Emma, Proclaim, Promec 20 EW, Vitex 50, Lepidex, Warlock 19.2 EC, Denim Fit,	Chloride channel activators, systemic and contact	6	Barley, dry bean, Cruciferae, groundnut, pea, potato, legume vegetables, maize, sweetcorn, sorghum, sunflower, soybean, wheat
Flubendiamide	Belt	Disruption of Ca2+ balance, non systemic	28	Endive, lettuce, maize, spinach and maize
Indoxacarb	Steward, Advance, Addition, Doxstar Flo	Voltage-dependent sodium channel blocker, contact	22A	cotton, Cruciferae, lettuce, maize, sweet pepper, sorghum, soybean, sweetcorn, potato, sugarcane, veldt, grazing
Indoxacarb / Novaluron	Plemax	Voltage-dependent sodium channel blocker, chitin inhibition, contact	22A and 15	Maize, sweetcorn
Lufenuron	Judge, Sorba,	Inhibitors of chitin biosynthesis, systemic	15	barley, dry bean, Cruciferae, groundnut, maize, pea, potato, sweetcorn, sunflower, soybean, sorghum, wheat
Mercaptothion	Avigard, Malathion, Avi-Merkaptothion DP,	Acetylcholinesterase (AChE) inhibitor, contact	1B	cotton, Cruciferae, groundnut, maize, sorghum, sugarcane, lawns
Methomyl	Spitfire 900 SP, Cyplamyl 90 SP, Masta 900 SP, Methomex 900 SP, Methomex 200 SL, Mylomex 900 SP, Methomate 200 SL	Acetylcholinesterase (AChE) inhibitor, contact	1A	cotton, Cruciferae, maize, potato, sorghum, veldt grazing,
Paridalyl	Sumipleo SC	Appears to inhibit insect vigour, contact	Unknown	Maize, sweetcorn
Profenofos	Farmag Profenofos 500	Acetylcholinesterase (AChE) inhibitor contact,	1B	Cotton, potatoes
Spinetoram	Delegate 250 WG	Nicotinic acetylcholine receptor (nAChR) allosteric activator, contact	5A	Cruciferae, maize, sweetcorn, sorghum
Spinetoram / Methoxyfenozide	Uphold 360 SC	Nicotinic acetylcholine receptor (nAChR) allosteric activator, contact	5A and 18	Maize, sweetcorn, sorghum

Many of the tested chemicals have increasingly become ineffective because of the repeated and widespread use of a limited range of insecticides with common active ingredients, against which the prevailing FAW biotypes have gradually gained resistance (Fatoretto et al. 2017). Resistance for the following class of chemicals: 1A (carbamates), 1B (organophosphates) and 3A (pyrethroids-pyrethrins) has been documented (Abrahams et al. 2017). The wide host-plant range, high reproductive capacity and rapid movement of FAW would most likely make the use of chemical insecticides non-durable and ineffective in the long run (Goergen et al. 2016). Furthermore, chemical pesticides have negative effects on the environment and some that are used in SSA are classified as highly hazardous pesticides (HHPs) (Bateman et al. 2018; Prasanna et al. 2018). As an interim approach, alternating pesticides based on their mode of action, and integrating these with non-chemical control measures, should decelerate the development of pesticide resistance (Abrahams et al. 2017; Stockad 2017). However, this is of no consequence for most smallholder farmers in Africa, who cannot afford modern insecticides, or the protective equipment used for safe handling of insecticides (Abrahams et al. 2017).

1.5.2.3 Biological control

Some biocontrol agents reportedly feed on or are toxic to FAW eggs or larvae, whereas others have been shown to prevent reproduction of FAW (FAO 2018a). In North and South America, several biological agents (bio-pesticides) have been used to control the FAW. These include *Telenomus remus* Nixon (Hymenoptera: Platygastridae), *Trichogramma chilonis* Ishi (Hymenoptera: Trichogrammatidae), *Orius insidiosus* Say (Hemiptera: Anthorcoridae) and *Doru luteipes* Scudder (Dermaptera: Forfuculidae) (Tefera, Goftishu, and Muniappan 2019; Souza et al. 2020). In Africa, *Telenomus remus* in Benin, Ghana, Cote d'Ivoire, Kenya, Nigeria and South Africa, and *Trichogramma chilonis* in Kenya, Benin and Ghana (Kenis et al. 2019; Tefera, Goftishu, and Muniappan 2019) were found to cause up to 45% parasitism against the eggs and larvae of the FAW. In Benin and Ghana, the most prevalent parasitoids, which had greater efficacy as biological control agents of egg-larval and larval parasitoids, were *Chelonus bifoveolatus and Coccygidum luteum*, respectively (Agboyi et al. 2020). Another novel FAW parasitoid, *Cotesia icipe* Fernández-Triana & Fiaboe (Hymenoptera: Braconidae), was reported in Kenya, Ethiopia and Tanzania (Sissay et al. 2018). Biopesticide production and use is a significant component of IPM strategies to effectively control FAW in Africa. In 2018, 29 biopesticide active ingredients were approved for use against lepidopteran pests in general, across 19 African countries (Bateman et al. 2018). Out of the 19 countries, South Africa was

the first to have FAW-specific biopesticides and the first three biopesticides registered for use are presented in Table1.4.

Table 1.4. Biological control agents first registered as microbial insecticides against Fall armyworm in South Africa †

Biocontrol agent	Brand name	Insecticide type	Crops
Bacillus thuringiensis var. *aizawai*	Florbac WG	Bioinsecticide	Maize, sweetcorn
Bacillus thuringiensis var *kurstaki* Strain SA-11	Delfin	Bioinsecticide	Cruciferae, barley, cotton, maize, sorghum, soybean, wheat.
Beauveria bassiana	Eco-Bb	Bioinsecticide	Cruciferae, maize, sweetcorn, soybean, tomato, sorghum

† (DAFF 2017)

1.5.2.4 Crop Plant Incorporated Protectants (PIPs)

A functional protein, Herculex® I, was co-developed by Dow Agrosciences and Pioneer Hi-Bred and has been shown to enhance resistance against FAW (Siebert et al. 2008). Bohorova et al. (1997) reported that among the Cry1-type δ-endotoxins specific for lepidopterans, Cry1D and Cry1F proteins derived from *Bacillus thuringiensis* were most toxic to *S. frugiperda* and some transgenic maize cultivars expressing these proteins are already in use in South Africa (ISAAA 2018). These toxic proteins are encoded by the *Cry 1* gene (Siebert et al. 2008). However, many SSA countries have not legally approved the use of transgenic crops (Schurman 2017). Coincidentally, FAW appears to have developed resistance to certain GM maize possessing the toxic proteins Cry1F, Cry1Ab and Cry1Ac (Ingber, Mason, and Flexner 2017), opening up further debate on whether to adopt GM technology in Africa. Nevertheless, Lourenco et al. (2017) reported an interesting synergistic effect of transgenic plants and silicate-fertilization in suppressing FAW damage in maize, but such a multi-dimensional approach needs further evaluation across several test environments.

1.5.2.5 Host-plant resistance

Several putative quantitative trait loci (QTL) associated with resistance to FAW in maize have been reported (Brooks et al. 2005). QTL associated with FAW leaf-feeding damage resistance

are listed in Table 1.5 (Brooks et al. 2005; Womack, Warburton, and Williams 2018; Womack et al. 2020). The reported QTL were localized on chromosome 9 and are ideal candidates for introgression into elite maize genotypes (Table 1.5). This will enhance development of maize breeding populations for FAW resistance in SSA. Maize genes conferring resistance to key insect pests include the *mir* gene family and the gene *gl*15, both of which confer resistance to South Western Corn Borer (SWCB) (Brooks et al. 2005). Some insect resistance genes confer horizontal resistance to multiple crop pests. However, the expression of QTL for FAW resistance depends on genotype, environment and genotype × environment (G×E) interaction. Thus, understanding the genetic basis of inheritance and the effect of the environment and their interaction in affecting host-plant resistance is key to exploiting candidate genes in breeding programs. There is a need to identify potential sources of host resistance in Africa and from areas where FAW was initially reported, such as the USA and Brazil, for introgression into African-adapted genotypes.

Table 1.5. Quantitative trait loci associated with reduced Fall armyworm leaf-feeding damage in maize

QTL†/Marker name/Position	Marker type	Chromosome number	Reference
LDR2	SSR‡	1	Brooks et al. (2005)
LDR4	SSR	2	Brooks et al. (2005)
LDR6	SSR	5	Brooks et al. (2005)
LDR9	SSR	6	Brooks et al. (2005)
LDR10	SSR	7	Brooks et al. (2005)
LDR11	SSR	9	Brooks et al. (2005)
LDR12	SSR	9	Brooks et al. (2005)
Zm.95692_212010250	SSR & SNP§	3	Womack et al. (2018)
umc1342	SSR & SNP	7	Womack et al. (2018)
PZA00498_5	SSR & SNP	8	Womack et al. (2018)
bnlg127	SSR & SNP	9	Womack et al. (2018)
PHM2828_83	SSR & SNP	10	Womack et al. (2018)
PZA01005_1	SSR & SNP	10	Womack et al. (2018)
69.3-83cM (Bin 4.05-4.06)	SSR & SNP	4	Womack et al. (2020)
56-62.8cM (Bin 9.03)	SSR & SNP	9	Womack et al. (2020)

† (QTL) Quantitative Trait Locus

‡ (SSR), Simple Sequence Repeats

§ (SNP), Single Nucleotide Polymorphism

1.5.2.6 Integrated FAW management

Integration of various compatible FAW-management strategies, viz., cultural practices, biocontrol and host-plant resistance, is internationally recommended. Host-plant resistance is

pivotal in integrated FAW management, especially for subsistence farmers (Abrahams et al. 2017; Prasanna et al. 2018). However, FAW-resistant or -tolerant cultivars have not yet been developed and deployed in Africa (FAO 2018a). The associated pros and cons of each control strategy, when used separately, suggest the need for an integrated FAW-management approach, which should be tested and deployed in SSA for effective management of the FAW. One important consideration when developing an integrated FAW-management program is whether there is compatibility and complementarity of the proposed control strategies between/among them.

1.6 Breeding maize for FAW resistance

Development of host-plant resistance through breeding is a core component of a comprehensive integrated FAW-management strategy. Two stages in this process can be distinguished; pre-breeding (genetic enhancement for FAW resistance) and actual breeding (cultivar development involving crosses using suitable FAW-resistant sources and subsequent selection). Pre-breeding can be used to identify the novel sources of resistance genes. Some common sources of FAW resistance and their origin for breeding are summarized in Table 1.6 (Abel et al. 2000; Brooks et al. 2005; Brooks et al. 2007; Miranda-Anaya, Guevara-Fefer, and Garcia-Rivera 2002; Ni et al. 2014; Prasanna et al. 2018; Widstrom et al. 1992; Wiseman et al. 1996). These genetic resources have been identified by the USDA-ARS and EMBRAPA research programs (Prasanna et al. 2018; Williams et al. 2018).

Table 1.6. Common sources of Fall armyworm resistance in maize and their origin

Name or code or pedigree	Source type	Reference
Antigua 2D x (B10x B14)	Tropical maize	Wiseman et al. (1996)
CRW3(S1)6	Tropical maize	Wiseman et al. (1996)
MP496	Temperate maize	Brooks et al. (2007)
GT-FAWCC (C5)	Tropical maize	Widstrom et al. (1992)
100-R-3	Tropical maize	Abel et al. (2000)
116-B-10	Tropical maize	Abel et al. (2000)
Mp704	Temperate maize	Brooks et al. 2005
Mp708	Temperate maize	Brooks et al. 2007
CML67	Tropical maize	Miranda-Anaya et al. (2010)
CUBA 164-1	Tropical maize	Ni et al. (2014)
DK7	Tropical maize	Ni et al. (2014)
UR11003:S0302	Temperate maize	Ni et al. (2014)
CML121-127	Tropical maze	Prasanna et al. (2018)

Modern cultivars, primitive or obsolete cultivars, landraces, wild relatives, breeding populations, and mutant varieties are potential sources of resistance that can be explored in FAW-resistance breeding (Mwadzingeni et al. 2017). Jessup et al. (2006) reported the potential of exploiting syntenic relationships between maize and other members of the Family Poaceae, such as Zoysiagrass (*Zoysia* spp. Willd.) and buffel grass (*Cenchrus ciliaris* L.), in which QTL for FAW resistance have been identified. Given the co-evolution of maize with FAW in Mexico, Peru and other South American countries, genes for FAW resistance can be found in maize germplasm collections from that region. Landraces and wild relatives are useful genetic resources for maize improvement for biotic and abiotic stress tolerance (Prasanna et al. 2018). Takahashi, Kalns, and Bernal (2012) reported that Mexican landraces possessed FAW-resistance genes that could be harnessed for developing FAW-resistant genotypes. Balsas teosinte (*Zea mays ssp. parviglumis*), a progenitor of cultivated maize and which has been used previously to improve agronomic traits, has been identified as a candidate source for FAW resistance (Takahashi, Kalns, and Bernal 2012). Given that gene flow and introgression of alleles commonly occurs between teosintes and cultivated maize landraces, teosintes can be explored as a source of FAW resistance in Africa.

The three key components in evaluation of maize for FAW resistance include: pest inoculum levels (number of larvae per plant or square meter), standardized infestation techniques, and damage rating/scoring. The most appropriate developmental stage of FAW larvae and suitable growth stage of maize should be targeted for successful infestation. Also, use of uniform larval stage should ensure effective infestation and repeatability of test results. A reliable source of infestation is essential for screenhouse or field evaluation of resistance in maize genotypes. This will ensure consistent levels of pest infestation, development and evaluation. Because of the migratory nature of the pest, field experiments should be preferably conducted in FAW hotspots. Laboratory rearing of the pest is a reliable source of FAW for screening experiments under controlled conditions. The insect-rearing process specifically requires careful management of adults, eggs, larvae and pupae, which requires diet formulation and dispensing (Prasanna et al. 2018).

A modified rating scale for FAW, adapted from Davis, Williams, and Wiseman (1989), is presented in Table 1.7. In the modified scale, categories A-F are introduced, which accommodate consecutive percentage ranges of all possible levels of FAW leaf-feeding damage between one and hundred percent. The use of percent scores rather than numeric scores of 1-9 takes into consideration the continuous nature of leaf-feeding damage caused by FAW

and the need for a linear scale to capture additional differences in leaf damage levels and hence to differentiate genotypes better. FAW infestation in the screenhouse involves guided application of larvae into the leaf whorl. The larval density during inoculation is dependent on plant stage. Typically, 2-3 larvae are used for seedling-stage evaluation, whereas 15-20 neonate larvae are used for the mid-whorl stage to achieve uniform FAW infestation (Ni et al. 2014). Damage assessment is conducted 7-14 days after infestation. Damage ratings should be taken in replications at 1-2-day intervals, for a week.

Table 1.7. Rating scale for assessing Fall armyworm damage in maize using percentage leaf-feeding damage †

Category	Leaf-feeding damage (%)	Equivalent score to Davis scale	Description
A	0-10	0-1	● Plants with no damage, windowing signs on leaves, up to five pinholes/leaf
B	11-20	2-3	● up to eight pinholes per leaf, ● up to five clearly visible shot holes per leaf ● one to two elongated lesions per leaf ● leaf whorls of one to three plants eaten up
C	21-30	4-5	● more than ten pinholes ● up to ten clearly visible shot holes ● 3-5 elongated lesions per leaf ● leaf whorls of up to five plants eaten up
D	31-40	6-7	● up to ten clearly visible shot holes per leaf ● up to five elongated lesions ● up to three clearly visible portions eaten away per leaf ● one to two dying areas on a leaf ● leaf whorls of more than five plants eaten up
E	41-50	8-9	● more than eight elongated lesions per leaf ● more than five portions visibly eaten away ● more than two dying portions on leaf ● leaf whorl almost entirely eaten (away dead heart with signs of new shoot)
F	>50	10	● dead heart with no signs of new shoot ● dying or dead plant ● severely damaged plant

† (Davis et al. 1989)

In SSA, FAW-resistance breeding is in its infancy. Most maize cultivars were found to be susceptible to FAW infestation/damage (Prasanna et al. 2018). There is a critical need to develop locally adapted FAW-resistant varieties through introgression of novel resistance genes, or the intensive accumulation of additive genes for resistance. To date, CIMMYT has been routinely screening adapted and temperate germplasm collections for resistance. This is

being done in partnership with the National Agricultural Research System (NARS) (Prasanna et al. 2018). In Brazil EMBRAPA is actively breeding for FAW resistance in maize, whereas IITA, CABI and ICIPE in SSA are developing comprehensive strategies to manage FAW through biocontrol techniques and other integrated approaches (http://www.iita.org/news-item/developing-strategy-combat-invasive-species/). FAO is engaged in capacity building and strengthening of monitoring systems for FAW presence (FAO 2018a). These are laudable collaborations that can provide new genetic resources and breeding tools for sustainable management of the pest through resistance and tolerance breeding. In September 2018, 35 national and international organizations formed the new Fall Armyworm R4D International Consortium led by CIMMYT. The coalition was aimed at developing technical solutions and a shared vision to manage FAW in SSA (https://agra.org/news/new-global-alliance-formed-to-fight-against-fall-armyworm/). Currently, most public breeding institutions in SSA are still conducting pre-breeding activities, including identification of sources of FAW resistance.

1.6.1 Genetics of FAW resistance in host plant

Genetic studies have shown that FAW resistance in maize is mostly polygenic in nature, and is controlled by both additive gene action and dominant gene action (Brooks et al. 2005; 2007). Understanding the underlying mechanisms of inheritance and the nature of gene action conditioning FAW resistance can be achieved through combining ability studies. Genetic designs, such as diallel, line × tester analysis and North Carolina Designs are effective in determining general and specific combining ability effects for FAW resistance through controlled-environment or field evaluations (Prasanna et al. 2018). In turn, the outcomes of studies point toward the primary mating designs that are appropriate to use for subsequent breeding for enhanced FAW resistance. If general combining ability (GCA) effect is the largest component, then the accumulation of additive genes is called for, using a form of recurrent selection. If specific combining ability (SCA) effect is pre-eminent, then a backcross program is called for, to introgress resistance genes into agronomically desirable parents. With a mixture of GCA and SCA effects in the parent population, recurrent selection to generate inbreds with accumulated additive genes may be followed by hybrid breeding to take advantage of the SCA effects. Williams et al. (2018) reported the significance of both GCA and SCA effects in the inheritance of FAW resistance, although GCA effects were pre-dominant. The existence of dominant gene action and epistatic gene effects indicates the potential to improve maize

resistance against FAW through hybrid breeding. Use of recurrent selection to accumulate additive genes should lead to the development of durable horizontal resistance against FAW. Breeding maize for FAW resistance should determine and validate the extent of GCA and SCA effects to formulate a suitable breeding strategy. When the SCA variance is significant, hybrid breeding approach should be invoked and crosses should be made between FAW-resistant and agronomically complementary elite inbred lines. If the GCA variance is significant, development of open-pollinated varieties (OPV) or population improvement methods, such as recurrent selection (RS) should be employed. Progeny selections with FAW resistance and desirable agronomic traits under multiple target production conditions should be practiced to fix minor genes. Given the affordability and ease of maintenance, OPVs are preferred by small-scale farmers in SSA. Another breeding approach is synthetic variety development through intercrossing of an improved population with a farmer-preferred local variety. In both breeding approaches, a large number of individuals should be maintained to minimize inbreeding.

1.6.2 Breeding methods for FAW resistance

Given the polygenic inheritance of FAW resistance, recurrent selection is ideal for accumulating additive genes (Abel et al. 2000). Widstrom et al. (1992) reported on the use of recurrent selection for FAW resistance and found that advanced cycles could serve as good sources of inbred lines with intermediate to high levels of resistance. Breeding for polygenic traits requires quantitative data, preferably from homogenous levels of pest infestations (Acquaah 2012).

1.6.3 Genomic selection (GS) for FAW resistance breeding

Application of genomic selection (GS) or genome-wide association study (GWAS) has the potential to enhance breeding of cultivars with a good balance of farmer-preferred traits (Bohra et al. 2014; Cantelmo, Von Pinho, and Balestre 2017). GS refers to selection decisions based on genomic breeding values (GEBV), calculated as the sum of the effects of molecular markers densely populated across the genome (Vishwakarma et al. 2016). Many genetic markers spread across the genome enable capturing of potentially all QTL contributing variation for a trait (Vishwakarma et al. 2016). Because of the ever-increasing replacement of traditional DNA-based markers by next-generation sequencing (NGS)-based markers, scientists can now

develop high density genetic linkage/QTL maps and decipher whole genome sequences of crops (Pandey et al. 2017). The amenability of NGS-based markers to high-throughput technology makes genome-wide selection through GWAS possible. GS has been applied in cereals, such as wheat and rice (Cantelmo Von Pinho, and Balestre 2017), and in legumes, such as groundnut, pigeon pea, chickpea and cowpea (Bohra et al. 2014), changing their status from "orphan" to "genomic-rich resources" crops (Varshney et al. 2013). Considering the potential of NGS, technologies, such as GWAS and RNA-sequencing to generate rich genomic resources for GS application, future collaborative strategies for FAW resistance research at a global level could consider integrating these platforms to enhance genetic gains.

1.6.4 Integrating breeding science and farmers' preferences

Adoption of new maize cultivars with FAW resistance and high yield can be enhanced through participatory plant breeding approaches. Maize breeders have previously focused on improving yield and yield-related traits. However, many new maize cultivars have been poorly adopted because farmer-preferred traits and farming circumstances were not considered in the breeding process. Participatory rural appraisal (PRA) is a multidisciplinary research approach, which has been adopted by some modern breeding programs. The PRA approach enhances farmer-breeder interactions and is one way of investigating the factors that influence farmers' choice of cultivar, farmer-perceived quality traits and other related factors (Mukanga et al. 2011). In the case of FAW management at the current outbreak, correct and accurate information is required to establish damage levels, actual yield losses and useful measures practiced on farmers' fields to reduce yield losses. Preliminary assessments of FAW-related crop damage based on farmer's perceptions has been reported in SSA (Baudron et al. 2019). However, early yield-loss assessments were inflated by respondent farmers, given farmers' limited knowledge and understanding of the pattern of damage caused by FAW on maize (Baudron et al. 2019). Effective PRA tools are required to collect reliable information on farmers' perceptions and indigenous knowledge systems for successful FAW resistance pre-breeding and breeding programs in SSA (Glover, Sumberg, and Andersson 2016).

1.7 Challenges, opportunities and future directions

FAW infestation has expanded from the Americas into Africa, and has now spread across most of SSA and into Asia. Challenges in managing FAW infestations are the absence of natural enemies/predators in Africa, the pace of FAW distribution, inaccurate forecasts and the pest's polyphagous behavior. FAW preference for maize makes it an economically important pest in Africa. Maize sustains millions of people in Africa directly or indirectly, and hence FAW affects household, national and regional food security. Remote sensing and effective weather forecasting techniques are needed for integrated FAW-management strategies. Of the proposed control strategies, host-plant resistance is the most viable and cost-effective long-term control option for subsistent farming communities; however, participatory breeding needs to be encouraged for considering farmer-preferred traits in breeding programs. Developing locally adapted landraces and OPVs, incorporating several minor genes for FAW resistance can be useful for small-scale maize farmers in SSA. Further, targeted crosses involving complementary parents can generate new breeding populations suitable for gene mapping, validation and introgression of QTL for sustainable FAW control. Transgenic maize could also be utilized as an effective management strategy. However, most countries in SSA have rejected the GMO approach. In addition, FAW has been reportedly building up resistance to *Bt* maize; thus, new non-*Bt* genes (both transgenes and cisgenes) should be utilized to circumvent *Bt* resistance problem. Public and private partnerships between various stakeholders, including FAO, CABI, CIMMYT, IITA, ICIPE, EMBRAPA, private-sector organizations, and especially farmers are key to the successful management of FAW in Africa. A combination of PRA-driven pre-breeding and hybrid breeding activities using conventional breeding techniques and NGS-based breeding is recognized as the most sustainable, long-term strategy to manage FAW in maize in Africa.

1.8 References

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CHAPTER 2. FARMERS' PERCEPTIONS OF PRODUCTION CONSTRAINTS AND THEIR TRAIT PREFERENCES IN MAIZE: IMPLICATIONS ON BREEDING FOR FALL AMYWORM RESISTANCE IN ZAMBIA

Abstract

The recent outbreak of the fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith (*Lepidoptera*: Noctuidae) has threatened food security in sub-Saharan Africa. The aim of this study was to assess farmer-perceived production constraints, trait preferences and implications of FAW invasion for maize cultivar development in Zambia. A participatory rural appraisal (PRA) study was conducted in two FAW affected districts in Zambia in 2017 and 2018 using semi-structured questionnaires, preference ranking and focus group discussions. High cost of fertilizers, limited agricultural land availability, insect pests, and drought stress were reported by 73%, 55%, 38% and 36.6% of the respondents, respectively, as the main production constraints. There were significant differences ($X^2 = 12.415$; $p = 0.002$) in the severity of FAW infestation between the two surveyed districts in 2016. Multivariate analysis for social, agronomic and plant protection-related aspects disaggregated by gender of respondent indicated that the first six principle components accounted for 69.6 and 68.7% of the variation among the male and female respondents, respectively. Prominent farmer-preferred traits were influenced by district, family size, insect resistance, grain maturity, drought tolerance and market price. Evidently, FAW resistance should be considered a key trait in addition to other traits such as drought tolerance in maize cultivar development and deployment in Zambia. Data presented in this study will serve as a basis to develop farmer-preferred maize varieties integrating FAW resistance in Zambia or related agro-ecologies in SSA.

Keywords: cultivar development, fall armyworm, farmer preferences, maize, participatory rural appraisal, Zambia

2.1 Introduction

Maize (*Zea mays* L., 2n = 2x = 20) is the most important cereal crop globally after wheat. Annually, over 1 billion tonnes of maize is produced from 193 million hectares (FAOSTAT 2018). The present global mean yield of maize is 5.9 t ha^{-1} (FAOSTAT 2018). Maize is suitable for low input production systems due to its genetic plasticity and adaptability to diverse agro-ecological conditions (McCann, 2005). The United States of America, China, and Brazil are the leading producers of maize. Sub-Saharan Africa (SSA) is the leading consumer of maize products with 121.87 g per capita per day (FAO 2017). In southern Africa, more than 300 million people depend directly on maize as a staple food (Macauley and Ramadjita, 2015).

In SSA, maize is mainly cultivated in small-scale and low input production systems (Diiro et al., 2018). These farming systems are characterised by major constraints including biotic stress (diseases and insects pests), abiotic stress (drought stress and infertile soils) and various socio-economic factors. Consequently, the present mean yield of maize in SSA stands at 2.1 t ha^{-1} (OECD 2018). Zambia is the second largest producer of maize in southern Africa after South Africa. In Zambia, maize is cultivated in all the three agro-ecological zones (AEZ I, II & III) over a total area of 1.2 million hectares (FAOSTAT 2018). The total agricultural land allocated for maize production in the country has increased over time following the reintroduction of strategic subsidy programmes such as the farmer input support program (FISP) (Mason et al., 2013). Maize yields in Zambia have remained low and stagnant with a mean national yield of 2.1 t ha^{-1} due to a combination of several production constraints, including insect pests and diseases (Chapoto, 2018; Chapoto and Subakanya, 2019). The major diseases of economic importance in Zambia include grey leaf spot caused by *Cercospora zeae-maydis*, northern corn leaf blight (*Exserohilum turcicum*), and common rust (*Puccinia sorghi*) (Mukanga et al., 2011). Maize production is also affected by major insect pests, which include weevils, stem borers, and termites (Kabwe et al., 2018) and recently the fall armyworm (FAW) (Abrahams et al., 2017).

The FAW [*Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae)] originated from the tropical and subtropical regions of the United States of America. The pest has over 350 hosts (Balla et al., 2019). In Zambia, FAW was first detected in December 2016 and has since spread quickly across the country, covering all the major agroecological zones by January 2018 (Kansiime et al. 2019). The FAW has reportedly caused extensive crop losses and affected natural ecosystems, biodiversity, and local, regional and global trade (FAO 2018).

Due to the recent outbreak of FAW there is no dedicated insect resistance breeding program to aid in the integrated management of the pest in Zambia. There is need to reassess maize production constraints, coping mechanisms, and farmers' trait preferences to guide breeding. The paucity of information in Zambia and elsewhere in Africa since the outbreak of the FAW in 2016 hinders the development of new maize cultivars that integrate farmers' and value chain preferences. Engaging the farmers and other stakeholders of the maize value chain in generating new information will augment current practices and replace obsolete knowledge for efficient breeding.

To investigate farmers' production constraints, circumstances, and initiate pre-breeding programs, participatory rural appraisal approaches (PRA) are recommended and have been widely used as the first step of market research (Mukanga et al., 2011; Daudi et al., 2018; Odendo et al., 2001). PRA are a bottom-up, fast-evolving family of methods that enable sharing, enhancing and analysing farmers' knowledge of their agricultural livelihoods and conditions, for the purpose of planning and action (Chambers, 1994). Mukanga et al. (2011) used PRA methods to investigate farmers' perceptions on maize ear rot and their implications on breeding for host plant resistance in Zambia. Comprehensively designed PRA methods provide a robust means of investigating farmers' trait preferences, production challenges prospects and priorities for crop breeding (Mukanga et al., 2011; Mrema et al., 2017). Inclusion of gender-responsiveness in the data collection and analysis process further enhances the robustness of PRA approaches in distinguishing inherent differences among different categories of the participants that may not be otherwise evident (Cornwall, 2003). In breeding maize for improved characteristics, farmer and market preferred traits must be included and in the case of FAW, this study is the first record soliciting farmers' inputs in Zambia. The objectives of this study were to 1) identify current constraints to maize production and assess the impact of the recent FAW outbreak in the different districts, 2) identify and rank the importance of farmer trait preferences and how they can be incorporated during breeding for FAW resistance in maize and 3) assess variation in perceived production constraints, trait preferences and knowledge of FAW across demographic groups and districts. The study is based on the following hypotheses 1) production constraints are heterogeneous across different districts, 2) gathering baseline information from consumers can help to inform breeders during breeding of cultivars with multiple desirable traits and increase cultivar adoption rates and 3) trait preference and perceived production constraints are gender sensitive.

2.2 Materials and methods

2.2.1. Description of study sites

The study was conducted in Central and Lusaka provinces of Zambia from Nov 2017 to April 2018 (Figure 2.1). These regions are situated in agro-ecological Region II that receive annual rainfall of between 800 and 1000 mm, which is distributed between November and April. The study region has variable soils that are largely classified as lixisols, luvisols, alisols, acrisols leptosols and vertisols. The sites were purposefully selected because they fall within a maize production area that was severely affected by the FAW outbreak since the 2016 cropping season (Kabwe et al., 2018). The study areas are characterised by moderately fertile soils with low incidences of nutrient leaching.

2.2.2 Sampling procedure

Study sites and farmers involved in maize production and affected by FAW outbreak were purposely sampled for the study. Two provinces (Central and Lusaka) were sampled, each represented by one district (Chibombo and Chongwe, in that order) (Figure 2.1). Two camps were sampled per district as follows: Nanswisa, and Chititi (Chibombo District) and Chainda and Chalimbana (Chongwe District). These provided four camps for the PRA study and in each camp, one village was sampled. A camp is the smallest unit of operation for extension delivery under the Zambian government extension service provision program. A group of camps forms a block and a group of blocks forms a district. In this study, 30 to 31 farmers, with first-hand experience and knowledge of FAW as revealed by prior situational analyses conducted in the target camps, were sampled per camp providing a total number of 121 farmers. These were interviewed using the structured questionnaires. Further, 25 farmers were sampled per camp giving a total number of 100 farmers for the focus group discussions (FGDs). Farmers who were involved in maize production for livelihood purposes and whose maize fields were damaged by FAW infestation were selected for both the questionnaire interviews and FGDs.

Figure 2.1. Map of Zambia showing the study areas highlighted by the black dots

2.2.3 Data Collection

Data collection involved the following stakeholders: farmers, a crop scientist, a social scientist, two extension officers from each district agricultural office, two senior agricultural research officers, two camp heads and four research assistants. The study was conducted through two transect walks and, two FGDs per camp, and a total of 121 face-to-face interviews using a semi-structured questionnaire. Transect walks were conducted in each camp and involved the participation of women, men and youth, for qualitative observations of farmers' cropping systems, the extent of FAW damage in farmers' fields and coping mechanisms. The FGDs involving situational analyses and preference ranking, disaggregated by demographics were conducted to gain insight into farmers' current circumstances, indigenous knowledge on pest management and opportunities for enhancing agricultural productivity. Farmers were divided according to demographic groups, which were defined by respondents' age, sex and their assigned roles and responsibilities within their communities for FGDs. Responses to discussion questions were sought from each of the three demographic groups. The semi-structured questionnaire was used to capture data on maize production practices, constraints, trait

preferences and the magnitude of FAW damage during the past two years. Prior to questionnaire administration, pretests were conducted with 10 farmers in each of the four camps to ensure quality data collection. Additional data on the socio-economic status, including educational level and income of the household head as the targeted respondent for the interview were collected through questionnaire interviews. The proportion of respondents who indicated an increased, decreased or constant land area devoted to maize production relative to the last four cropping season (2016-2017) was regarded as indicative of maize production trends in the surveyed areas.

2.2.4. Data Analysis

The Statistical Package for Social Sciences (SPSS) version 24 (IBM Corp 2016) was used to analyse all questionnaire data. Descriptive statistics and frequencies were deduced, while pair-wise comparisons between groups of respondents were cross-tabulated and subjected to Pearson Chi-square test statistic to deduce trends and validate their significance for decision-making. Factors influencing maize trait preferences based on gender and age of respondents were subjected to principle component analysis following the dimension reduction procedure using a correlation matrix on SPSS. The major trends emanating from qualitative data obtained during the FGDs were used to substantiate the quantitative data obtained from the questionnaires.

2.3 Results

2.3.1. Socio demographic characteristics of the sampled farmers

The number of males and females did not differ significantly between districts (X^2 =0.002; p =0.962) (Table 2.1). There were significant differences in the age (X^2 =22.56; p =0.000) and family sizes (X^2 =9.953; p = 0.007) of the respondent farmers within and between the districts. The respondents were aged between 15 and 70 years old and their family sizes ranged from 1 to 15 family members. The level of education between the two districts did not differ significantly (X^2 = 0.003; p = 0.768). The majority of the farmers in both districts had attained basic primary education. Differences in household income between Chibombo and Chongwe was non-significant (X^2 = 0.005; p = 0.562) with most farmers earning between Zambian Kwacha (ZMW) 3000 – 5000 annually. Seventy percent of the sampled households owned agricultural land ranging between 1 to 5 hectares (Table 2.1) and farm size varied across districts with significantly more farmers in Chongwe owning farms between six and 15

hectares. A small proportion (8.4 %) of the respondents in Chibombo did not own land but either rented in or shared lands with other farmers.

Table 2.1. Socio-demographic profile of respondent farmers (%) in Chibombo and Chongwe districts obtained through face-to-face interviews. (N=121)

Variable	Class	Chibombo	Chongwe	Chi-square	Df	p-value
Sex	Male	31.7	30.8	0.002	1	0.962
	Female	19.2	18.3			
Age (years)	15-30	19.1	1.7	22.56	3	0
	31-50	20	19.1			
	51-70	11.3	20.9			
	>70	1.7	6.1			
	Zero	6.4	5.9			
Educational level	Primary	21.3	27.2	0.003	3	0.786
	Secondary	14.3	11.5			
	Tertiary	8	5.6			
Family size (number)	<5	16.4	4.3	9.953	2	0.007
	5-10	31	38.8			
	>10	3.4	6			
Household income (ZMW)	<1000	3.6	5.2	0.005	3	0.562
	1000-3000	17.4	19.3			
	3000-5000	18.5	15.7			
	>5000	10.5	9.9			
Land owned (ha)	0	8.4	0	14.706	5	0.012
	<1	2.5	0.8			
	1-5	34.5	37			
	6-10	4.2	8.4			
	11-15	0.8	2.5			
	16-20	0.8	0			

Note: df, degrees of freedom; Primary and secondary education refer to grades 1 to 7 and 8 to 12 respectively, while tertiary education refers to a college certificate/ diploma or university degree; ZMW, Zambian Kwacha

2.3.2 Maize production systems in Chibombo and Chongwe districts

Respondent farmers estimated that over the past four years, maize yields in the survey areas ranged between 2.12 and 3.14 t ha $^{-1}$ (Table 2.2). On average, Chibombo had higher maize yield between the two districts. The overall mean production area devoted to maize per household in the study districts was 1.75 ha. Chainda camp had the highest average area under maize production per respondent of 2.07 ha, while Chalimbana camp, with 1.33 ha, had the lowest. In Chongwe district, 87.5 % (44.6 and 42.9 % for Chainda and Chalimbana camp, respectively) of the respondents acquired seed from cooperatives. Only farmers in Chibombo's

Nanswisa camp obtained seed from Zambia Agricultural Research Institute (ZARI) office, although the majority of respondents (56.6%) in this camp sourced their maize seed from agro-dealers. Maize was grown largely as a sole crop in Chibombo district.

Table 2.2. Maize production trends and systems at camp and district levels during the 2017/18 survey season.

Camp/district	Production area and farmers' perception of trend of maize production				Cropping system		Main source of seed			Use of fertilizer and crop rotation		Mean yield (tonnes/ha) ± SD
	Mean ± SD cultivated area per household for past 4 seasons (ha)	Relatively increased compared to past 4 years (%)	Relatively constant compared to past 4 years (%)	Relatively decreased compared to past 4 years (%)	Sole crop (%)	Inter-crop (%)	Cooperatives (%)	ZARI (%)	Agro-dealers (%)	Mineral fertiliser (%)	Crop rotation	
Camp												
Nanswisa	1.90 ± 1.87	16.7	45.8	37.5	100.0	0.0	1.2	63.1	35.7	26.3	28.8	3.14 ± 4.23
Chititi	1.73 ± 1.89	38.6	32.8	28.6	100.0	0.0	65.6	0.0	34.4	29.5	16.3	2.89 ± 3.43
Chainda	2.07 ± 0.83	35.5	12.0	52.5	25.4	79.8	72.8	0.0	27.2	16.8	26.0	3.05 ± 2.09
Chalimbana	1.33 ± 0.82	44.2	45.7	10.2	34.8	65.2	65.9	0.0	34.1	27.4	28.8	2.12 ± 2.76
District												
Chibombo	1.82 ± 1.87											3.02 ± 3.83
Chongwe	1.69 ± 0.90											2.57 ± 2.48
Mean	1.75											2.8

Note: SD = standard deviation; ZARI, Zambia Agricultural Research Institute.

2.3.3. Constraints to maize production in the study areas

During the questionnaire interviews, respondent farmers described 13 major constraints affecting maize production and productivity in the study areas (Table 2.3). High cost of fertiliser was ranked as the most important constraint to maize production by 73.0% of the farmers. The second and third most important constraints were insect pests and limited agricultural land. Other very important production constraints included drought stress (reported by 36.6% of respondents) and poor soil fertility (28.6%) and lack of suitable traits in the newly released varieties (7.7%).

Of the 73.0% respondents that ranked high cost of fertiliser as a very important constraint, 35.0% from Chibombo and 37.9% from Chongwe concurred with the ranking. Insect pests were considered as very important or of intermediate importance to maize production, by between 31.0 and 38.0% of the respondents. Insect pests including the fall armyworm, weevils, termites and stock borers were the most common and challenging insect pests in the study area. Most farmers classified poor soil fertility (42.9%) as a constraint of intermediate importance. Varieties lacking desirable traits was ranked as the lowest constraints with overall ranking of 10 and classified as having intermediate importance by 69.2% of the farmers in the two districts. Another important constraint was limited access to fertilizer, 25.8% of the respondents, respectively.

Table 2.3. Major constraints to maize production as reported by the farmers during the face-to face interviews in the survey areas.

Constraints	Overall rank (%)	Level of importance	Chibombo (%)	Chongwe (%)	Total (%)	Chi-square	df	p-value
Limited agriculture land	13.5 (3)	VI	20.7	6.1	26.8	41.167	3	0
		IM	23.2	2.4	25.6			
		II	7.3	9.8	17.1			
		NI	11.0	19.5	30.5			
Poor soil Fertility	9.2 (5)	VI	14.3	14.3	28.6	17.685	3	0.039
		IM	26.8	16.1	42.9			
		II	10.7	3.6	14.3			
		NI	10.7	3.6	14.3			
Low-yielding varieties	3.0 (9)	VI	0.0	5.6	5.6	5.056	3	0.537
		IM	27.8	33.3	61.1			
		II	16.7	0.0	16.7			
		NI	5.6	11.1	16.7			
Limited access to improved varieties	3.1 (8)	VI	5.3	0.0	5.3	10.556	3	0.307
		IM	5.3	10.5	15.8			
		II	21.1	10.5	31.6			
		NI	15.8	31.6	47.4			
New varieties lacking suitable traits	2.1 (10)	VI	7.7	0.0	7.7	4.494	3	0.343
		IM	0.0	0.0	0.0			
		II	7.7	61.5	69.2			
		NI	7.7	15.4	23.1			
A lack of improved seed	5.0 (7)	VI	6.7	0.0	6.7	16.815	3	0.052
		IM	10.0	16.7	26.7			
		II	6.7	40.0	46.7			
		NI	10.0	10.0	20.0			
High cost of fertilizers	17.0 (1)	VI	35.0	37.9	72.8	19.86	3	0.019
		IM	6.8	16.5	23.3			
		II	3.9	0.0	3.9			
		NI	0.0	0.0	0.0			
Limited access to fertilizer	5.1 (6)	VI	22.6	3.2	25.8	41.361	3	0
		IM	29.0	25.8	54.8			
		II	12.9	3.2	16.1			
		NI	3.2	0.0	3.2			
Drought Stress	11.7 (4)	VI	7.0	29.6	36.6	35.515	3	0
		IM	1.4	1.4	2.8			
		II	18.3	22.5	40.8			
		NI	16.9	2.8	19.7			
Insect pests	15.2 (2)	VI	19.6	18.5	38.0	28.287	3	0.001
		IM	5.4	21.6	31.5			
		II	7.6	3.3	10.9			
		NI	8.7	10.9	19.4			
Diseases	5.0 (7)	VI	3.3	0.0	3.3	22.585	3	0.007
		IM	20.0	20.0	40.0			
		II	13.3	6.7	20.0			
		NI	10.0	26.7	36.7			
Bird damage	3 (9)	VI	0.0	11.1	11.1	3.651	3	0.455
		IM	0.0	0.0	0.0			
		II	22.2	27.8	50.0			
		NI	27.8	11.1	38.9			

Note: df, degrees of freedom; ns, non-significant; VI, very important; IM, important; II, intermediate important; NI, not important.

2.3.4. Farmer's traits preferences in maize

Farmers listed and ranked several traits that they preferred in a maize variety during the questionnaire interviews (Table 2.4). The trait preferences showed highly significant differences between the two districts (X^2 = 33.8; p = 0.000) and these were associated with maize production, marketing and consumption. About 57 and 42% of the respondents ranked high yield as the most important and preferred trait from Chibombo and Chongwe, respectively. Insect pest resistance, ranked by 64% of respondents from Chibombo district was the second highest preferred trait after disease resistance in that district. In Chongwe district, drought tolerance, and suitability for intercropping were prioritized by 65 and 58% of the farmers. Other traits included cooking quality, low aflatoxin accumulation and good market price that were ranked highly by the farmers in Chibombo district, while processing quality, early maturity and best adaptability were ranked highly by ≥ 50% of farmers from Chongwe district.

Table 2.4. Trait preferences in maize by farmers in Chibombo and Chongwe district

	District				
Trait	Chibombo (%)	Chongwe (%)	Chi-square	Df	p-value
High yield	42.3	56.8	33.8	11	0
Processing quality	46.0	54.0			
Cooking quality	61.0	39.0			
Suitability for intercropping	42.0	58.0			
Early maturity	46.9	53.1			
Drought tolerance	34.5	65.4			
Insect resistance	64.3	35.5			
Disease resistance	75.0	25.0			
Storage pest resistance	52.5	47.5			
Low aflatoxin accumulation	60.0	40.0			
Good market price	55.5	44.5			
Best adaptability	50.0	50.0			

df, degrees of freedom

2.3.5. Factors influencing farmers' trait preferences in maize

The first six principal components (PCs 1 to 6), with eigen values greater or equal to 1 accounted for 69.57% and 68.74% of the variation in farmers' trait preferences in maize explained by the respondents' gender, respectively (Table 2.5). PC1 accounted for 21.46% and 18.96%, while PC2 accounted for 12.82% and 13.70% of the variation attributable to gender of the respondent, respectively. Respondent's district and family size loaded highly as factors under PC1, while PC2

had access to extension services and good market price as high-loading factors. For the female gender, early maturity and drought tolerance loaded highly under PC1 and PC2, respectively.

Table 2.5. Eigen values, proportions of social, agronomic and plant protection factors influencing farmers' preferred traits in maize

Factors	Male						Female					
	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6
Eigen values	3.01	1.8	1.59	1.23	1.11	1.01	2.66	1.92	1.62	1.25	1.15	1.03
Proportion of variation	21.46	12.82	11.33	8.81	7.93	7.21	18.96	13.7	11.55	8.95	8.19	7.38
Cumulative variation	21.46	34.28	45.61	54.42	62.35	69.57	18.96	32.66	44.21	53.16	61.35	68.74
Social												
Region	0.906	0.116	-0.019	-0.012	-0.017	-0.139	0.93	-0.072	-0.007	0.057	0.139	0.033
Extension advise	0.005	0.649	-0.214	-0.67	0.305	0.273	0.136	0.594	-0.368	-0.114	-0.006	-0.305
Family size	0.543	-0.225	-0.142	0.108	0.056	0.349	0.476	0.319	0.135	0	-0.395	-0.111
Good market price	0.255	0.621	0.296	0.223	0.121	0.309	0.429	0.611	0.069	0.04	-0.276	-0.287
Agronomic												
High yield	-0.221	-0.055	0.065	0.59	-0.238	-0.427	-0.206	0.099	0.134	0.579	-0.125	0.42
Early maturity	0.376	0.451	0.201	0.282	-0.288	-0.22	0.506	0.48	0.103	0.214	-0.086	0.292
Drought tolerance	-0.456	0.381	0.448	-0.001	0.26	-0.119	0.322	0.608	0.324	-0.085	0.091	-0.135
Best adaptability	0.025	0.078	0.026	-0.804	-0.152	-0.194	0.017	-0.006	-0.044	-0.745	0.281	0.38
Cooking quality	0.025	0.018	-0.812	0.078	0.115	-0.224	-0.029	-0.208	-0.733	0.257	0.301	-0.04
Plant protection												
Insect resistance	-0.106	-0.649	0.571	0.025	0.177	0.131	-0.23	-0.419	0.733	-0.014	-0.067	-0.235
Disease resistance	-0.011	-0.247	-0.219	0.234	0.683	-0.075	-0.219	-0.151	-0.044	0.361	0.38	-0.455
Storage pest resistance	-0.339	-0.111	-0.363	0.174	-0.468	0.537	-0.384	-0.096	-0.344	0.107	-0.52	0.255

2.3.6 Frequency and severity of FAW occurrence in Chibombo and Chongwe districts

All the interviewed farmers in Chibombo and Chongwe reported the occurrence of FAW in their maize fields in at least one season during the 2016-2017 and 2017-2018 crop seasons (Table 2.6). There were significant differences (X^2 = 12.415; p = 0.002) in the severity of FAW infestation between the districts, although the severity did not differ significantly (X^2 = 4.469; p = 0.298) after multiple infestation, in the main and off-season. Based on a single season infestation by FAW, 58.3% respondent farmers reported moderate damage to their maize fields, while 79% of respondents with multiple seasons of infestation reported severe crop damage.

Table 2.6. Occurrence and severity of FAW in the study areas

Frequency of occurrence, symptoms, and control methods of FAW	Description	District		Total	Chi-square	Df	p-value
		Chibombo	Chongwe				
Occurred in one season (main season)	SD	13.9	1.4	15.3	12.415	2	0.002
	MOD	34.7	23.6	58.3			
	MID	6.9	19.4	26.4			
Occurred in two seasons (main and off seasons)	SD	33.3	46.2	79.5	1.082	1	0.298ns
	MOD	12.8	7.7	20.5			

Df, degrees of freedom; ns, non-significant; SD, severe damage; MOD, moderate damage; MID, mild damage

2.3.7. Farmers' perceptions of FAW damage symptoms, severity and associated yield loss

Table 2.7 presents FAW damage symptoms, severity and associated yield loss as perceived by farmers in the study districts. There were highly significant differences (X^2 = 17.626, p = 0.001) in FAW damage symptoms reported by farmers from the two districts, whose maize fields experienced severe FAW damage (Table 2.7). About 61% of these farmers observed substantial leaf damage, while 19.5% reported changes in the seed shape as the most common symptoms of FAW damage on maize. These farmers estimated that yield loss due to severe damage by FAW was above 50%. For farmers whose maize field experienced moderate and mild FAW damage, the observed damage symptoms were similar. The most common damage symptom under moderate and mild FAW damage was leaf colour change, which was observed by 68 and 75% of farmers, respectively. Change in seed shape was reported by 16% of the farmers under moderate FAW damage and 10% under mild FAW damage. Across all damage levels, the differences among the observed FAW damage symptoms were highly significant (X^2 = 22.057, p =0.001). Overall, farmers described leaf colour change and 'big leaf portions eaten away' as the most observed symptoms reported by 48 and 30.6% of respondents,

respectively. Figure 2.2 shows a typical FAW damaged maize crop at the Chainda camp in Chongwe District in February, 2018.

Table 2.7. Level of FAW damage, associated symptoms and yield loss in maize as perceived by the respondent farmers in Chibombo and Chongwe districts

		Observed symptoms by district						
Level of damage	Farmers' perception of yield loss	Symptom	Chibombo (%)	Chongwe (%)	Total (%)	Chi-square	Df	*P*
Severe	50% <	large leaf portions eaten away	17.1	43.9	61.0			
		Seed shape change	19.5	0.0	19.5			
		Leaf colour change	9.8	2.4	12.2	17.626	4	0.001
		Seed size change	4.9	0.0	4.9			
		Shot holes in leaf	0.0	2.4	2.4			
Moderate	20- 49%	Leaf colour change	38.0	30	68.0			
		Seed shape change	16.0	0.0	16.0	7.145	3	0.067
		Big leaf portions eaten away	8.0	6.0	14.0			
		Shot holes in leaf	0.0	2.0	2.0			
Mild	<20%	Leaf colour change	15.0	60	75.0			
		Seed shape change	5.0	5.0	10.0	3.81	3	0.283
		Big leaf portions eaten away	5.0	5.0	10.0			
		Spotted leaf	5.0	0.0	5.0			
Total		Leaf colour change	23.4	25.2	48.6			
		Big leaf portions eaten away	10.8	19.8	30.6			
		Seed shape change	15.3	0.9	16.2	22.057	5	0.001
		Seed size change	1.8	0.0	1.8			
		Shot holes in leaf	0.0	1.8	1.8			
		Spotted leaf	0.9	0.0	0.9			

Df, degrees of freedom

Figure 2.2**.** A maize crop damaged by FAW at Chongwe's Chainda camp in Zambia.

2.3.8. Farmers' knowledge of control methods against FAW in Chibombo and Chongwe Districts

Farmers reported seven control methods that can be used to mitigate the adverse effects of the invasive FAW in maize (Figure 2.3). Application of sand/ash in maize leaf funnels was the most common control method reported by 28.3 % of farmers. The use of chemical pesticides was the second most common control method and was reported by 24.8% of farmers, while the use of resistant varieties was the least reported control method, reported by only 2.7 % of the farmers.

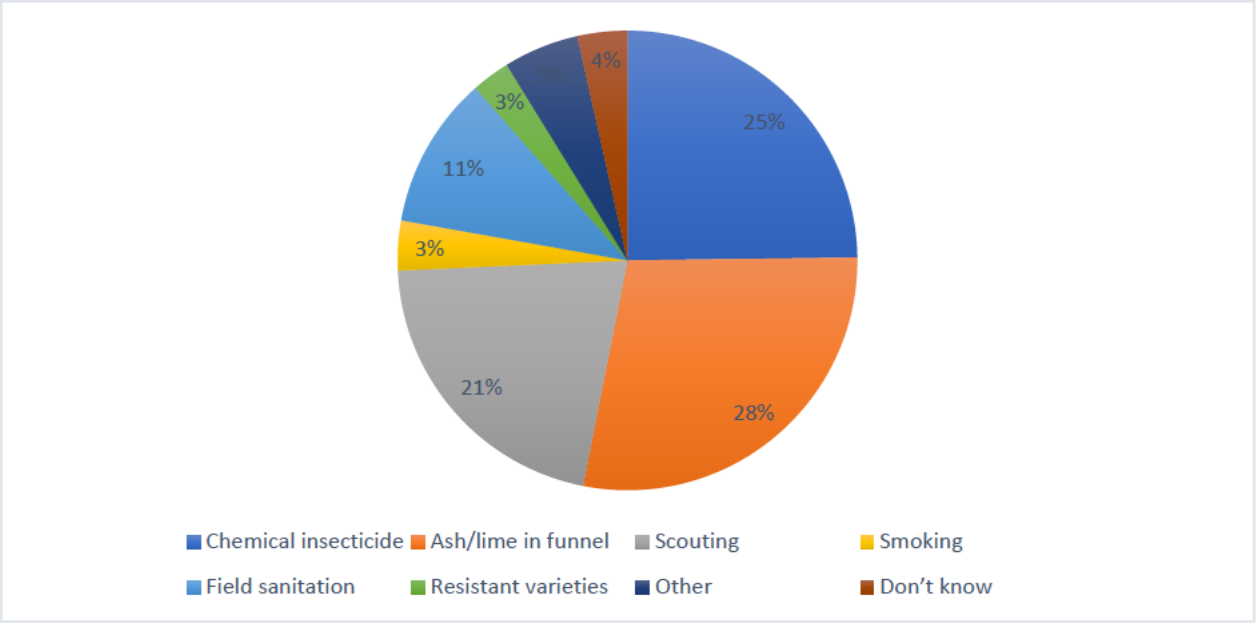

Figure 2.3. Farmers' perceptions of effective control methods against FAW.

2.4 Discussion

2.4.1. Socio demographic profile of farmers

There were non-significant differences between the numbers of participating males and females between Chibombo and Chongwe districts (Table 2.1). This indicates a similarity in the gender-responsiveness of the two districts with regards to maize production practices. Although women play significant roles in maize production (CGIAR 2002), the men were the ones mostly interviewed as the household heads and the ultimate decision makers on the use of the household's resources. Diiro et al. (2018) also highlighted that even in male-headed families in Africa, women and children played substantial roles in maize production systems. Participants in the FGDs indicated that maize is often regarded as a man's crop because of its role in both household food security and income generation, both of which are roles traditionally associated with men in most rural communities. Doss (2002) reaffirmed that maize is perceived as a man's crop, especially in SSA. Despite this perception, maize production was evidently inclusive of all demographic groups in both districts, as observed during the transect walks and FGDs. For instance, FGDs revealed that women and youth were responsible for land preparation, weeding, fertilizer application harvesting, seed sorting and packaging, while the men were responsible for input procurement, pesticide application, and the identification of markets and transportation of produce to the depots for sale. The involvement of all demographic groups shows that maize is an important crop in this area.

2.4.2. Characterization of the maize production systems

Maize production systems, farm sizes and land ownership have implications on the ability to control the FAW, the actual control methods that can be used and scouting ability. Farmers with large farm sizes will most likely have more available casual labour and inputs that can also be applied for FAW control since the cultivation of bigger land portions typically requires a larger labour force. Given the high cost of chemical inputs and the general scarcity of resources among the smallholder farmers (Chapoto and Subakanya, 2019), effective control of FAW using recommended pesticides was not available among the farmers. Scouting for FAW in large plots by farmers can be inefficient leading to failure to detect FAW early enough for effective intervention. Farmers with small-sized farms were more vulnerable because their crop could suffer complete decimation even under typical SSA FAW infestation. The currently susceptible varieties grown by the farmers in the region exacerbate the impact of FAW damage (Kasoma et al., 2020). Farmers in Chibombo district (Nanswisa and Chititi camps) completely practiced sole cropping, which provided the FAW availability of host plants on a wide scale. The lack of alternative cropping predisposes the farmers to potential food insecurity if the monocrop of maize is completely destroyed. Crop rotation with alternative crops such as soya beans and groundnuts could reduce the impact of FAW damage on maize (Muzangwa et al., 2017), but only a few of the farmers (29.0%) practiced rotations (Table 2.2).

2.4.3. Constraints to maize production

Farmers' ranked high cost of commercial fertilizer as the most important constraint to maize production in agreement with (Mukanga et al., 2011) who also identified high cost of fertilizer and other production inputs among the major factors curtailing maize production by small-scale farmers in SSA. High cost of fertilizer and the inability of the farmers to apply optimal fertilizer means that the crop suffers from prevalent nutrient deficiencies exacerbating the impact of FAW. Incorporating nutrient-use efficiency, while breeding against FAW would be advantageous for the farmers. Further, the use of organic fertilizers could be explored as many farmers in the survey areas reared goats. Drought stress compromises the ability of crops to withstand other biotic or abiotic stress. Crops with sub-optimal nutrition and moisture availability are unhealthy and have compromised ability against pests. The ranking of insect pests among the production constraints shows that the farmers realise the impact of insect pests on yield and food security. Previously, yield was the integral trait preferred in maize production among farmers prior to FAW invasion. Mukanga et al. (2011) had indicated that insect pests

were a minor constraint of maize production in Zambia, particularly in the central province, which was also part of this investigation. However, insect pests, especially the FAW have only recently gained in importance as a major constraint to maize production in Zambia, and SSA generally (Prasanna et al., 2018). Thus, insect pests will likely continue to feature as a high-ranking constraint to maize production in the region, until sustainable methods of control or resistant varieties are developed. The prevalence of other pests and diseases (as mentioned by 31-38% of respondents), other than FAW, also contributes to the impact of FAW either as secondary pests or acting in combination with more devastating effects. With recent changes in climate patterns and FAW outbreaks, farmers realise that resilience is also an important trait. High yield potential is ideal under optimal climatic and agronomic conditions, but the recurrent challenges means that farmers would rather have resilient cultivars with lower yield potential but reasonable yield under adverse conditions to ensure food security. The lack of access to improved and high yielding varieties means that the farmers continue to cultivate landraces or low yield potential cultivars. Such cultivars expose the farmers to possible food insecurity even under mild or low infestation that would otherwise have little impact on high yielding cultivars. It is essential that breeding programs take recent events in environmental changes, pests and disease epidemics, and end-user preferences into cognisance during product development.

2.4.4. Frequency and severity of FAW occurrence in Chibombo and Chongwe districts

Farmers whose fields experienced FAW infestations over two successive cropping seasons reported more severe damage to their maize fields compared to the damage reported by farmers whose fields were only infested during one season. This trend may be attributed to the latter's limited experience with the FAW pestilence resulting in undue attribution of FAW damage symptoms to other pests or diseases. Coincidentally, the presence of the FAW may have evaded the farmers since the FAW larvae burrow into the leaf-whorl during the day and only come out at night or very early in the morning when most farmers are away from their fields (Hruska and Gould, 1997). This observation could be pertinent when FAW occurs during seedling stage and the FAW damage could be attributed to a stem cutter (Mihn, 1983). On the contrary, FAW infestations were reported to be more severe by farmers with more than one FAW season's experience because the pest established more stable populations by the second season and, therefore, had greater potential to cause severe damage. This assumption is supported by the findings of (Rosenweig et al., 2001), that insect populations spawn in successive seasons, increasing the severity of crop damage especially in warm temperatures. Successive

infestations also increased the farmers' ability to distinguish FAW damage from other pests. The non-significant differences between the two districts suggested that there was a gradual build-up of FAW populations in subsequent seasons, spurred by favourable environmental conditions and availability of suitable hosts. This could indicate prospects for incorporating crop rotation in IPM strategies. The frequency and severity of FAW shows that it is a devastating pest and is projected to increase as it has numerous alternative hosts such as perennial grasses and cultivated cereals, posing a real threat to food security in Zambia and SSA if sustainable control methods are not developed urgently (Prasanna et al., 2018).

2.4.5. Factors influencing farmers' trait preferences in maize

Multivariate analysis of the factors influencing farmers' trait preferences in maize (Table 2.5) revealed that social factors followed by a combination of agronomic and plant protection-related factors ranked highly. The factors that loaded highly for PC1 were mainly of social and gender- responsive nature, suggesting that gender and other social factors are important considerations when assessing the impact of production constraints and designing appropriate breeding programmes. For instance, in general, males in Africa are not involved in food processing at home, so the cooking quality of maize is not a priority trait among male farmers (Kemmer, 2000). Males would prefer high yield for economic returns and insect resistance to reduce costs associated with crop protection and labour hours. In contrast, females are mostly involved in food preparation and would prefer maize with good cooking quality. While the females would also prefer high yielding maize to ensure adequate food supply, they are less interested in insect and drought tolerance as costs and labour associated with crop protection are generally male dominated (Ochago, 2018). Thus, we speculate that female-owned fields could be more vulnerable to FAW and other constraints due to lack of economic means to control the FAW and a lack of particular attention to cultivation of resistant cultivars. The PC2 had high contribution from a combination of mainly agronomic and plant protection-related factors and thus could be regarded as an axis for plant intrinsic factors to guide maize breeding programs. Drought tolerance was among the agronomic factors contributing to PC2, which corroborated with (Kassie et al., 2013), who reported that farmers in Southern Africa frequently and consistently prefer varieties with improved drought tolerance. Improving drought tolerance, pests and disease resistance will improve productivity under farmer conditions. It will also improve adoption rates of cultivars by farmers thereby impacting positively on food security. Indirectly, drought tolerance will also improve the crop's ability to withstand

secondary infectious diseases or reduce the impact of yield loss due to FAW. Resistance to insect pests also loaded highly in PC2, confirming that insect pest damage could be the second highest constraint that the farmers faced in the PRA sites. Improvement in pests and disease resistance mentioned by the farmers is important. However, the farmers do not specify the exact pest or diseases that are prevalent in their fields or areas. While the FAW is a recent outbreak, it is important to include it in the portfolio of pests that must be targeted for crop improvement. Other pests must also be taken into consideration to offer a wider and more stable spectrum of resistance. Previously, high yield, disease resistance, earliness, white grain colour and processing qualities have been listed as leading traits preferred by farmers in maize (Sibiya et al., 2013). Mukanga et al. (2011) listed ear rot resistance as the most important trait preferred by maize farmers in central and southern Zambia. Changes in farmers' trait preferences and constraints over time and place reflect shifts in the challenges and opportunities for agricultural research and development in SSA that can be addressed most suitably through a demand-led breeding approach. One advantage of demand-led breeding is the integration of shifting market trends and drivers, enabling accurate forecasting of cultivar adoption and therefore, ensuring value for investment in plant breeding (Shimelis, 2017). Improved adoption rates for superior cultivars will improve maize productivity and positively impact on household and national food security statuses.

2.4.6. Farmers' perceptions of FAW damage symptoms, severity and associated yield loss

A large proportion of farmers who reported that they observed large leaf portions eaten away and estimated yield loss to be more than 50 % showed that the majority of the farmers were only able to identify FAW field infestations too late. None of the farmers could identify the early larval feeding damages of "windowpane" and shot holes in leaves. Two groups of farmers, those who incurred severe damage and those who suffered mild crop damage due to FAW, did not observe the shot holes or pinholes characteristic at the onset of FAW infestation. When coupled with the misidentification of pest damage symptoms, failure to identify early FAW damage symptoms reveals an opportunity to strengthen awareness on the importance of famers' participation in pest monitoring. This finding affirms the important role that farmers could play in field monitoring for FAW infestation to enable early scouting and identification to prevent significant yield losses. Prasanna et al. (2018) recommended field scouting as a monitoring exercise in which farmers should participate to enable timely identification of FAW for effective control. The most reported symptom of leaf-colour change by famers with

moderate and mild damage to their maize is not a documented sign of FAW damage in maize and may therefore be associated with other factors not related to FAW. This affirms that farmers may fail to distinguish FAW damage when the crop suffers from a plethora of other pests and diseases, which could confound efforts to combat relatively new challenges such as FAW outbreaks. Moreover, the mild to moderate damage in some fields may be attributed to the ability of maize plants to recover from injury and compensate for growth in some cases. Damage recovery and growth compensation in maize that previously suffered FAW damage was observed in earlier studies (Wiseman and Davis, 1979). Baudron et al., 2019 established that perceived maize yield loses in SSA due to FAW may be overrated owing to the pest's feeding pattern of leaf-tattering, that leaves a hopeless appearance to an otherwise recoverable maize field. For effective prevention and control of FAW, there is need to strengthen the role of farmers in monitoring, farmer education and improvement in extension services in response to outbreaks. The ability to identify FAW invasion and its symptoms could also be helpful in identifying sources of genetic resistance to the pest for breeding purposes.

2.4.7. Farmers experience and coping strategies with insect pests

Knowledge of the use of resistant cultivars in the control of pests and diseases was only reported by ~3% of the respondents suggesting a lack of information dissemination on pests and disease control strategies among the small holder farmers and also shows that the farmers would not ask to procure improved cultivars that they did not know about. Most farmers indicated during the FGDs that they were aware, through regular extension services that FAW resistant maize varieties were not yet available for use in the country. Therefore, most farmers resorted to the use of ash/sand applied to the leaf funnel in an attempt to control FAW especially in Chongwe district, where farmers indicated that they did not have reliable access to chemical pesticides. Desperate farmers who formulated their own unorthodox pesticides by dissolving detergent paste in water sometimes burnt their crops unwittingly. Although some of the farmers were of the perception that the ash/sand and detergent methods were effective in controlling FAW, further research is required to investigate the origin of these methods and whether they could be effective at a large scale. Mihale et al. (2009) report several indigenous knowledge-based methods used by subsistence farmers in the management of field and storage pests including lepidopterans that closely resemble the FAW. Farmers' management methods ranged from the use of animal products such as cow dung and urine to plant parts including leaf-oil extracts and husks, some of which are under study as FAW biocontrol strategies (Abrahams et

al., 2017; FAO 2018). Some of the strategies mentioned by the farmers are only appropriate under small plots e.g. the application of sand/ash in whorls and detergent spraying. The effectiveness of these strategies against FAW is not validated and may not be effective leading to the spread of FAW and its negative impact on yield and food security Harrison et al., 2019). The recent outbreak of FAW has compounded the effects of chronic production constraints in SSA with catastrophic impact on maize production and food security. Farmers lack effective FAW control strategies and they have limited knowledge of the pest, which hinders FAW control efforts that depend on early identification of infestation. Developing FAW resistant cultivars will contribute towards an integrated FAW management strategy. Coupled with farmer-preferred traits, the incorporation of FAW resistance during cultivar development will increase adoption rates for improved cultivars and contribute to increase in maize production and strengthening of food security statuses of many households. However, further research is required to accurately assess FAW associated yield losses in maize in order to dispel misinformed perceptions and enable more accurately informed decisions by policy makers and other stakeholders in ensuring food security in SSA.

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CHAPTER 3. SCREENING OF INBRED LINES OF TROPICAL MAIZE FOR RESISTANCE TO THE FALL ARMYWORM AND FOR YIELD AND YIELD-RELATED TRAITS

Abstract

The fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith) is a major cause of crop loss and food insecurity in sub-Saharan Africa. There is a need to identify maize genetic resources with FAW resistance for breeding. The objectives of this study were to screen two sets of diverse maize germplasm and to select genotypes with FAW resistance and desirable yield-related traits. Set I and Set II, containing 60 and 253 maize genotypes, respectively, were selected based on their agronomic potential and adaptability for screening in FAW infested areas in Zambia. Highly significant differences ($P< 0.001$) were detected among test genotypes for FAW-leaf damage (FLD) and FAW cob damage (FCD) and agronomic traits. The lowest FLD and FCD were 8.87% and 5.36%, recorded in genotypes CML304-B and CML442, respectively. Five principal components (PCs) accounted for ≥80% of the total variation showing that key traits included reduced anthesis-silking interval (ASI), plant height, FLD and FCD, desirable ear aspect and grain yield. Some genotypes, such as Pool 16 and ZM7114 from Set I, and CZL1310c, CML444-B, CZL15220 and TL1512847 from Set II had low mean FCD and FLD, showing that they were potential sources of FAW resistance. Grain yield was negatively correlated with mean FLD ($r= -0.18$, $p<0.05$), and FCD ($r= -0.15$, $p<0.05$). Promising maize genotypes including CZL1310c, CML444-B, CZL15220 and TL1512847 and CML491 were selected for their low mean FLD, FCD, earliness to flowering and high grain yield potential. These genotypes will be useful in developing tropical and sub-tropical maize breeding populations with partial FAW resistance and yield gains.

Keywords: fall armyworm, genetic diversity, inbred line, landraces, maize, partial resistance, phenotyping[2]

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3.1 Introduction

Maize (*Zea mays* L., 2n = 2x = 20) is one of the most important cereal crops in the world (FAOSTAT, 2016). Since its introduction in Africa in the 16th century (Miracle, 1965), maize has become a staple crop, providing over 30% of annual caloric intake to more than 300 million people in the region (Macauley and Ramadjita, 2015). The importance of maize in sub-Saharan Africa (SSA) is reflected by its wide variation in most traits, including yield, grain attributes (grain type, size, colour, quality, and texture), maturity period, pest and disease resistance, drought tolerance, and micronutrient content (Ekpa et al., 2018). However, maize production and productivity in SSA is consistently challenged by biotic and abiotic constraints, which threaten the food security and economic stability of the region. Among biotic stresses, the arrival of the invasive fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith) in Africa in 2016 and its spread to various parts of the continent contributed to major crop losses and food insecurity. FAW is a rapidly multiplying, gregarious insect pest and damages all the above ground plant parts of susceptible hosts, especially cereal crops, with yield losses varying from 17 to 43% depending on the infestation pressure and environmental conditions (Hruska and Gould, 1997).

Countries in North and South America have registered synthetic pesticides with various modes of action (Abrahams et al., 2017). However, many of these chemicals have become ineffective against FAW due to pesticide resistance (Fatoretto et al., 2017). Insecticides are too expensive and inaccessible to be considered by many smallholder farmers in SSA and Asia. Cultural practices such as adjusting planting dates, crop rotation, burning of crop residues and removal of alternate hosts around maize fields are essential for FAW control but may not be adequate under smallholder farming systems (Abrahams et al., 2017). The use of natural enemies such as parasitoids, entomopathogenic fungi and nucleopolyhedroviruses has shown potential in controlling FAW (FAO, 2018). However, these natural enemies are either available in short supply naturally or are only commercially available, making them too expensive for smallholder farmers in SSA. Genetically modified (GM) maize varieties expressing *Bacillus thuringiensis* (*Bt*) toxins can also be used to control FAW. Siebert et al. (2012) reported that GM maize expressing Cry1F or Cry2Ab2 + Cry1A.105 incurred less FAW damage than non-Bt maize varieties. Transgenic maize varieties expressing the Cry2Ab2 + Cry1A.105 proteins had less FAW related foliar damage when evaluated under sub-Sahara African conditions (Botha et al., 2019). However, some FAW biotypes have developed resistance to CryF1, Cry1A.105 and Cry2Ab2 (Huang et al., 2014; Omoto et al., 2016).

Each of the control strategies mentioned above has limitations when used alone. Ideally, an integrated approach is needed for long-term, sustainable management of FAW across SSA for both commercial and smallholder production systems. This approach should be tested and deployed in SSA for effective management of FAW under both commercial and smallholder production systems. Host plant resistance, the plant's intrinsic ability to resist or tolerate FAW herbivory, is an economic, sustainable and environmentally friendly approach that can be deployed to both smallholder and commercial farmers in SSA. The integration of host plant resistance has the potential to improve the effectiveness of FAW management programs (Farias et al., 2014; Abrahams et al., 2017; Kumela et al., 2018) however, commercial maize cultivars with resistance to FAW are not yet available in Africa.

There is a need to screen diverse genetic resources and to identify promising maize genotypes for FAW resistance breeding (Prasanna et al., 2017). Evaluating diverse germplasm will identify superior genotypes from a wider pool of genetic material to improve specific traits of interest. Early-stage phenotyping should involve a large number of genetically diverse genotypes to increase the probability of identifying favourable genotypes and achieving strong selection response (Ghanem et al., 2015). Evaluating diverse maize germplasm including landraces, improved varieties, open pollinated varieties (OPVs) and synthetic varieties, which are products of intercrossing between elite genotypes with known superior performance such as inbred lines or open pollinated varieties, could assist in identifying parental germplasm with wide genetic potential for FAW resistance.

Landraces with diverse genetic background and local adaptation, and elite maize germplasm and breeding lines have been collected and developed by international organizations such as the International Maize and Wheat Improvement Centre (CIMMYT) and the International Institute for Tropical Agriculture (IITA) as well as national breeding programs such as the Zambia Agricultural Research Institute (ZARI). These genetic resources contribute to a large pool of genetic diversity that exists in southern African maize, which should provide the required genetic variation for crop improvement. Therefore, there is a need to search for FAW resistant maize genetic resources for resistance breeding and as a basis for the integrated management of the pest in the region. Therefore, the objective of this study was to screen diverse maize germplasm and select promising genotypes with superior levels of FAW resistance, yield, and yield-related traits for breeding.

3.2 Materials and methods

3.2.1 Study site description

The study was conducted at the Mount Makulu Research Station (15°32.87S; 28°14.92E, altitude 1225m) in Zambia. The site is in agro-ecological region II. The region receives an annual rainfall of 800-1000mm with a long-term average of 765mm. The site is characterised by well drained, deep, yellow-red clay soils of pH 5.7 with medium base saturation. Since the outbreak of FAW in Africa in 2016, Mount Makulu has been observed as one of the areas in Zambia with consistently high FAW infestations including during the off-season, which is suitable for screening of maize for FAW resistance under natural infestations.

3.2.2 Plant materials

Two sets of studies were conducted. In Set I, 60 maize genotypes, were evaluated, which included landraces, hybrids and open pollinated varieties (Table 3.1). This study was intended to optimize the FAW evaluation and scoring method based on the present test conditions and following Davis et al. (1989). This study was conducted during the summer crop-growing season of December 2017-April 2018. In Set II, 256 maize genotypes were screened consisting of 253 tropical and sub-tropical inbred lines sourced from CIMMYT. Three local commercial hybrids were also included as comparative controls (Table 3.2). This study was conducted during the off-season (July to December 2018).

Table 3.1. Set I maize genotypes used in the study

Genotype	Province of collection/ releasing authority	District of collection	Target production region in Zambia	Days to maturity	Remarks	Reference
ZM 5021 ZM 4234 ZM 5043	Central/ZARI	Kabwe Mumbwa Serenje	II	135-140	Early-medium maturing	NPGRC
ZM 4358, ZM 4329, ZM 4327, ZM 4318, ZM 4325, ZM 4316, ZM 4323 ,ZM 4310, ZM 4312, ZM 4237, ZM 4307, ZM 4324, ZM 4336, ZM 4337 ZM 4333 ZM 4321	Eastern/ZARI	Chipata Lundazi Katete	II	135-145	Early-medium maturing	NPGRC
ZM 4321 ZM 4353, ZM 4342 ZM 3676	Muchinga/ZARI	Katete Chama Isoka	II	130-140	Medium maturing	NPGRC
ZM 4748	North -Western/ZARI	Kabompo	III	130-140	Late maturing	NPGRC
ZM 4236, ZM 4264, ZM 4258 ,ZM 4250, ZM 4252 ZM 4253, ZM 4252-1 ZM 4261, ZM 4245, ZM 4256 ZM 4235 ZM 4271	Southern/ZARI	Choma Monze Kalomo Namwala Sinazongwe	I	115-125	Early maturing	NPGRC
MM 400 MM 441 MM 501 MM 502 Pool 16	ZARI	-	I&II I&II I&II II&III I&II	110-120 110-120 120-130 135-145 100-120	Old varieties	Masole and Gumbo (1994); Howard and Mungoma (1996)
ZMS 606	ZAMSEED	-	I & II	125-130	Popular hybrids in Zambia	Smale et al. (2013); Waldman et al. (2016); Mubanga et al. (2018)
ZMS 638	ZAMSEED	-	I & II	125-130		
DKC 9089	Monsanto	-	I & II	115-125		
DKC 8053	Monsanto	-	I & II	120-130		
DKC 777	Monsanto	-	I & II	120-135		
DKC 8033	Monsanto	-	I & II	100-115		
SY 5944	MRI-Syngenta	-	II & III	120-130		
Pan 7M- 83	Pannar Seed	-	III	135-145		
PHB 30G19	Pioneer	-	II	135-145		

NPGRC- National Plant Genetic Resources Centre (http://www.fao.org/pgrfa-gpa-archive/zmb/nfp html)/Zambia; ZARI - Zambia Agricultural Research Institute; MRI-Syngenta Maize Research Institute-Syngenta seed company;-= Not available; Regions I, II and III denote Agro-ecological regions I, II and III of Zambia, respectively

Table 3.2. Set II maize genotypes used in the study

Genotype	Source	Target production region	Remarks	Reference(s)
CML443, CML543, CML572, CML571, CML546, CML197-B, CML312-1, CML395, CML440-B, CML441-B, CML442, CML444, CML445, CML548-B, CML495, CML489, CML491, CML494, CML536, CML537, CML538-1, CML539, CML540-B, CML545-B, CML545-B, CML546-B, CML547-B, CML548-B, CML547, CML548©, CML538-2 CML304-B, CML312-2	CIMMYT- Zimbabwe	Tropical and sub-tropical	Released CIMMYT derived inbred lines (CML)	Wu (2016); Masuka et al.(2017); CIMMYT (2019)
CML444-55, CML491-71, CML494-74, CML494-76, CML494-78, CML537-106, CML537-102, CML444-176, CML539-116, CML539-114, CML539-113, CML537-108, CML451-215, CML486-216, CML496-222, CML144-246	CIMMYT- Zimbabwe	Tropical and subtropical	CIMMYT lines derived from released CMLs (444, 491,494,537,539	–
CZL147013, CZL16084, CZL16017, CZL15006, CZL16090, CZL16091, CZL16092, CZL16093, CZL16095, CZL16098, CZL1609, CZL16100, CZL16101, CZL16043, CZL16045, CZL15142, CZL15142, CZL15220, CZL15103, CZL15178, CZL16170, CZL1423, CZL16173, CZL1349, CZL16121, CZL16067, CZL1606, CZL16138, CZL16139, CZL16026, CZL15168, CZL16107 ,CZL16106, CZL16049, CZL15076, CZL1508, CZL151301, CZL16051, CZL15192, CZL15194, CZL16061, CZL16063, CZL16080,CZL16075, CZL16032, CZL16027, CZL16029, CZL16016, CZL1463, CZL16184, CZL15234, CZL15181,CZL16132, CZL15119,CZL1346, CZL1368, CZL1347, CZL1350, CZL16025, CZL1360, CZL16013, CZL13104, CZL15123, CZL1466, CZL13100, CZL16143CZL15128, CZL15127, CZL15077, CZL15083, CZL1432, CZL1358, CZL15209, CZL16134, CZL16147, CZL15222, CZL15224, CZL16181, CZL15228, CZL16015, CZL15122, CZL15225, CZL15231, CZL16141, CZL15237, CZL15205, CZL16136, CZL16137, CZL0710, CZL15033, CZL1310c, CZL04016, CZL055011, CZL1117, CZL1011c, CZL16176 , CZL16177, CZL068, CZL15173, CZL16084, CZL15202, CZL0712, CZL1369, CZL111, CZL1354, CZL1211, CZL03011, CZL052	CIMMYT- Zimbabwe	Tropical and sub-tropical	Advanced breeding lines	Wu et al. (2016); Masuka et al. (2017); CIMMYT (2019)
CKDHL0228, CKDHL0323, CKDHL0089-B, CKL05024	CIMMYT- Zimbabwe	Tropical and sub-tropical	CIMMYT Kenya lines,	–
EBL169550, EBL173764, EBL173777, EBL1611442, EBL173776, EBLI611449, EBL1611440, EBL173778, EBL1611436, EBL173810, EBL1611462, EBL1611469, EBL167726	CIMMYT- Zimbabwe	Tropical and subtropical	CIMMYT early breeding lines,	–
TL1611607, TL1316, TL1611603, TL123332, TL1611615, TL1611611, TL1512861, TL116067, TL1611608, TL1611613, TL139178, TL139180, TL155952, TL122165, TL116004, TL139251, TL148348, TL115741, TL115743, TL139200, TL145733, TL1611609, TL115786, TL1512841, TL1512845, TL1512846, TL148266, TL1611610, TL1611604, TL145671, TL12176, TL115679, TL115627, TL139155, TL133972, TL142036, TL1512849, TL132023, TL142151, TL142139, TL142140, TL14217, TL1421839, TL1512864, TL1512869, TL1611554, TL1512847, TL131755, TL142054, TL1512886, TL1512887, TL1512891, TL13159, TL142017, TL142012, TL1313, TL1315, TL141998, TL173, TL155942, TL101644, TL116163-1, TL116163-2, TL123268, TL156610, TL156611	CIMMYT Zimbabwe	Tropical and sub-tropical	CIMMYT lines derived from CML and other backgrounds	–
VL05617, VL054530, VL056281, VL05614, VL058553, VL081463, INTA-181, LaPosta--141, SYN312-236	CIMMYT- Zimbabwe	Tropical and sub-tropical	CIMMYT lines, derived from assorted background	–
Check 1- MM 502	Agro-dealer/Zambia	II & III	ZARI released hybrid	–
Check 2- PHB 30G19	Agro-dealer/Zambia	II	Pioneer released hybrid	–
Check 3-DKC 777	Agro-dealer/Zambia	I & II	Monsanto released hybrid	

Tropical climate refers to warm to hot climate with mean annual temperatures above 18 C; sub-tropical climate is a relatively warm to hot climate with average temperature of 22 C in the warmest month; - = Not available; CIMMYT- International Maize and Wheat Improvement Center

3.2.3 Experimental design and crop establishment

The experiment involving Set I was laid out in a 6 × 10 alpha lattice design with three replications. Each genotype was planted in a two-row plot of 3m in length with 0.30m between plants and 0.75m between rows. A net plot size of 4.5 m^2 (hereafter referred to as a plot) was used for each genotype per replication. Three seeds were planted per hole and the seedlings were thinned to one plant per hole two weeks after emergence. Fertilizer application and weeding were carried out as recommended for maize production in Zambia (Mueller and Sisson, 2013). The crop was grown under natural rainfall, and supplementary irrigation was provided to avoid drought stress when necessary.

Set II experiment was laid out in a 16 × 16 alpha lattice design with two replications. Single row plots of 3m in length were used. Field planting and agronomic management were as described above. The genotypes were grown under rain fed conditions but supplementary irrigation was used when necessary to prevent the confounding effects of drought stress. Both trials were conducted at the Mount Makulu Research station under natural FAW infestations.

3.2.4 Data collection

3.2.4.1 Assessment of agronomic traits

The common agronomic traits measured in both Set I and Set II trials were plant height, days to anthesis and silking, anthesis-silking interval, fresh weight and grain yield. Plant height (PH, expressed in centimetres) was measured during reproductive stage 1 (R1), when one or more silks had extended out of the husk leaves, on five representative plants from each plot using a measuring tape from the ground to the top of the tassel. Days-to-anthesis (DTA) and days-to-silking (DTS) were recorded as the growing degree day units (GDDU) from planting to the day when 50% of the plants in a plot had produced pollen and silk, respectively. The (GDDU) were calculated according to Mueller and Sisson (2012) as follows;

$$GDDU = \sum_{n1+n2....nn} \left(\frac{T_{max} + T_{\min}}{2}\right) - T_b$$

Where,

T_{max} and T_{min} represent the daily maximum and minimum ambient temperatures, respectively. T_b represents the base or threshold temperature for maize at 10˚C(Soler et al., 2005) and n represents the number of days for anthesis or silking when the mean daily temperature was above the base temperature for the period from the effective date of planting to anthesis/silking.

Anthesis-silking-interval (ASI) was expressed as the difference in GDDU between DTS and DTA. Fresh weight (FW expressed in kg) was the total weight of all unshelled ears harvested from a plot measuring 4.5 m^2. Grain yield was calculated after adjusting moisture content to 12.5% as follows:

$$GY=[FW * \left(\frac{10000}{Plot\ Area}\right) * \left(\frac{(100-GM)}{(100-12.5)}\right) * SP]$$

Where, FW is fresh weight; GM is the grain moisture percent of sample as measured by a handheld moisture meter (Dickey John MINI GAC 1, USA); SP is the shelling percentage calculated as the (GW/FW)*100, where GW stands for grain weight; GY is the grain yield expressed in t ha^{-1}.

Ear aspect (EASP) and ear rot (ER) were only measured for the Set I trial in addition to the common traits. EASP was visually rated on a scale of 1 to 5, where 1 = clean, uniform, large, and well-filled ears and 5 = rotten, variable, small, and partially filled ears. Ear rot (ER) was recorded at harvest as the number of ears showing signs of ear rot infection per 4.5 m^2 plot, expressed as a percentage of the total number of ears harvested from each plot.

For Set II, the final number of plants at harvest (FNP), total number of cobs (NC) and number of well-filled cobs (WFC) were recorded in addition to the common traits. FNP was the number of plants in a plot at harvest, NC was recorded as the number of cobs obtained from a plot, while the WFC represented the number of cobs within a plot that had attained ≥ 50% grain filling.

3.2.4.2 Assessment of FAW variables

In both Set I and Set II trials, three pheromone traps consisting of the universal bucket trap, Russell IPM's four-component lure (Russell IPM, Flintshire, United Kingdom) and an insecticide strip were stationed 30m from the trials during the vegetative growth stages. The lures were changed three times during the growth period at four-week intervals and were used to collect weekly data on adult FAW moths (data not presented) as an indication of FAW pest pressure in the area.

Two key FAW parameters were recorded as FAW leaf damage (FLD) and FAW cob damage (FCD) for the two sets of evaluations. Both Set I and Set II evaluations used the rating scale of Davis et al. (1989) with modifications. A new rating system was developed in this study to avoid the limitations of an ordinal scale, as used by Davis et al. (1989). Briefly, the procedure

used in Set I involved careful examination of 10 randomly sampled plants per 4.5m^2 plot for FAW feeding damage. Each of the 10 plants were assigned a class based on the percent leaf area damaged by FAW. The number of plants in each class was then multiplied by the median damage percent of that class and the resultant values were added and divided by 10 to provide the final percent rating of a genotype per plot (Appendix 3.1). This scale ensures a robust assessment of FAW damage based on several plants across replications and variable growth stages rather than a generic plot-based assessment. Ratings from Set I evaluation were used to inform and optimize the FAW damage assessment protocol that was used during Set II evaluation. As a result, only two FLD scores were used in Set II after the others were found to be redundant based on the Set I evaluation trial.

In Set I evaluation, FLD values were recorded on 10 separate sampling dates between the vegetative growth stages3 and 8 (V3 to V8) and designated as FLD1 to FLD10. FLD was assessed every 2 days after the first FAW infestation was observed in the field. This was done to determine the most suitable growth stage and interval for FAW assessment under the local climatic conditions since there are no documented methodologies for FAW damage assessment in Zambia. The best scoring stages were determined by monitoring progressive foliar damage and identifying the FLD at which the highest significant differences among the test genotypes occurred. The ideal scoring interval or frequency for leaf damage was determined by considering consecutive FLDs that were significantly different from each other as revealed by paired t-tests (data not shown). The first FLD record was taken 14 days after the first signs of FAW damage in the field and referred to as FLD1. Thereafter, FAW damage was recorded at two-day intervals on days 16, 18, 20, 22, 24, 26, 28, 30, 32 after the first FAW infestation during Set I genotype evaluations (FLD2 - FLD10).

In Set II, FLD assessments were conducted twice only and designated as FLD1 and FLD2. The first FLD assessment was carried out 16 days after the initial FAW infestation was observed, owing to the slow development of inbred lines compared to the test landraces. This was followed by the second FLD assessment, which was conducted six days after the first one.

In both sets of evaluations, FCD was recorded at harvest as the number of ears per 4.5 m^2plot showing ≥50 % FAW induced ear damage expressed as a percentage of the ears in each plot. A value of 50% was considered to be a threshold for discriminating the genotypic susceptibility after careful observation of the continuous variation in cob damage.

3.2.5 Data analyses

The data collected were assessed for normal distribution and homogeneity of variance using the Shapiro-Wilks test procedure before analyses of variance were performed for both trials. The mean FLD was calculated from FLD1 to 10 in Set I and used to calculate the area under pest progress curve (AUPPC). Further, pest damage progress for each genotype in Set I was determined through the calculation of AUPPC for each genotype using the formula adapted from Henrichs and Miller (1991) and Jeger and Viljanen-Rollinson (2001) described below.

$$AUPPC = \sum_{i=1}^{n=1} \left[\left(\frac{FLD_i + FLD_{i+1}}{2} \right) (t_{i+1} - t_i) \right]$$

Where,

FLD_i represents the mean of the ith FLD across the three replications, beginning with FLD1 to FLD10

Y_{i+1} represents the mean the ith FLD plus 1

t_i represents the ith time point at which leaf damage assessments were made, beginning with 14 days through to 32 days after the first signs of FAW infestation

t_{i+1} represents the ith time point plus 1

Data on FAW leaf damage, anthesis-silking-interval, FAW cob-damage, field weight and ear rot were subjected to analysis of variance using an alpha lattice procedure in Genstat version 18 (Payne, 2015). Based on the FLD mean values, genotypes were categorised into low resistance (<15 %, equivalent to a score of <3 based on Davis et al. (1992) scale), moderate resistance (15-20%, a score of 3 to 4) and high resistance (>20%, a score > 5) classes using a genotype performance approach (Bertan et al., 2007). Pearson correlation analysis was performed to describe the pairwise association among agronomic traits. Multivariate analysis for the FAW damage and maize agronomic traits was performed to determine genotype response and association of traits in R (R Core Team, 2017). Figures describing the distribution of test genotypes with respect to the measured traits were produced using "FactoMineR" version 1.42 in R studio (Husson et al., 2019).

3.3 Results

3.3.1 Area under pest progress curve (AUPPC) for Set I evaluation

The AUPPC values of the Set I genotypes ranged from 383.00 to 541.67 with the genotypes DKC 9089 and ZM 4342 having the lowest and highest values, respectively (Table 3.3). For genotypes Pool 16 and ZM 7114, which had low AUPPC values, pest damage during the vegetative stage progressed relatively slower and comparable to levels of pest damage in the hybrids DKC 9089, DKC 777 and DKC 8053. The progress of pest damage in genotypes ZM 4342, ZM 4261 and ZM 4312, which had high AUPPC values, was relatively faster and comparable to the progress exhibited by the hybrid PAN7M-83.

Table 3.3. FAW leaf damage scores and area under pest progress curve (AUPPC) values for the top fifteen and bottom five performing genotypes ranked by AUPPC values in Set I evaluation

Top fifteen genotypes											
Genotype	FLD1 (%)	FLD2 (%)	FLD3 (%)	FLD4 (%)	FLD5 (%)	FLD6 (%)	FLD7 (%)	FLD8 (%)	FLD9 (%)	FLD10 (%)	AUPPC*
DKC 9089	9.33	14.00	15.17	19.33	20.67	23.33	25.33	24.33	26.67	36.00	383.00
Pool 16	10.87	12.67	15.96	23.33	23.33	23.33	23.33	23.33	26.93	30.47	385.78
ZM 7114	11.33	11.00	11.67	20.33	22.67	23.33	23.83	25.33	32.67	42.33	395.33
DKC 777	9.00	10.33	14.00	22.50	25.17	26.33	28.00	28.00	28.00	28.00	401.67
ZM 4358	12.00	12.00	13.33	23.00	26.67	25.33	25.33	26.83	29.00	31.33	406.33
ZMS 606	14.83	14.83	14.83	19.00	20.33	24.00	24.67	26.67	33.67	36.67	407.50
MM 501	9.00	10.67	16.67	21.50	23.67	25.33	28.67	28.67	32.00	37.67	421.00
MM 502	13.00	13.00	13.00	19.00	22.17	24.33	29.17	29.17	35.83	37.00	421.33
ZM 4249	9.67	10.83	16.33	22.33	23.00	28.33	28.33	28.33	32.67	32.67	422.67
ZM 4308	9.00	12.33	15.83	23.33	23.33	23.33	28.33	29.00	34.00	35.33	423.33
ZM 4253	13.33	14.17	14.17	21.67	22.50	27.33	27.33	27.33	33.83	35.33	425.33
ZM 4318	11.67	14.67	14.67	25.00	25.67	25.67	25.67	25.67	32.33	35.67	426.00
ZM 5021	13.67	13.67	14.67	24.67	24.67	25.67	26.67	29.83	31.00	31.67	427.00
ZM 4236	8.00	11.67	12.00	18.67	22.00	25.67	31.00	31.00	38.33	38.33	427.00
PHB 30G19	11.33	16.50	16.50	22.67	24.33	25.33	27.33	30.33	30.33	30.33	428.33
Bottom five genotypes											
ZM 4312	12.67	18.67	22.17	30.00	31.67	31.67	31.67	31.67	34.67	45.67	522.67
ZM 4271	18.33	18.33	18.33	29.00	29.00	29.67	34.33	34.33	41.00	41.00	527.33
ZM 4264	21.00	21.00	21.00	30.33	30.33	32.00	32.00	32.00	38.00	40.17	534.50
ZM 4261	16.33	16.33	21.00	26.17	31.67	31.67	33.33	37.00	44.00	44.00	542.67
ZM 4342	19.00	19.00	23.00	27.00	28.17	33.67	33.67	33.67	43.00	48.33	549.67

Note: FLD1 refers to the first FAW leaf damage rate recorded 14 days after pest initiation to FLD10 the last FAW leaf damage recorded 32 days after pest initiation during Set I evaluation. *the genotypes were ranked according to AUPPC values.

There was a trend of a gradual increase in leaf damage from FLD1 to FLD10 among the selected genotypes with low (Figure 3.1A) and high (Figure 3.1B) resistance in Set I. The AUPPC for the representative high and low resistance genotypes ranged from 9.0 - 42.3% and

12.7- 48.3%, respectively. The final leaf damage rating (percent damage at FLD10) and the AUPPC values were indicative of the variable reaction of the test genotypes to FAW. Of the ten representative genotypes, DKC 777 and ZM 4342 had the lowest and highest FLD10 values of 28.0 and 48.3%, respectively.

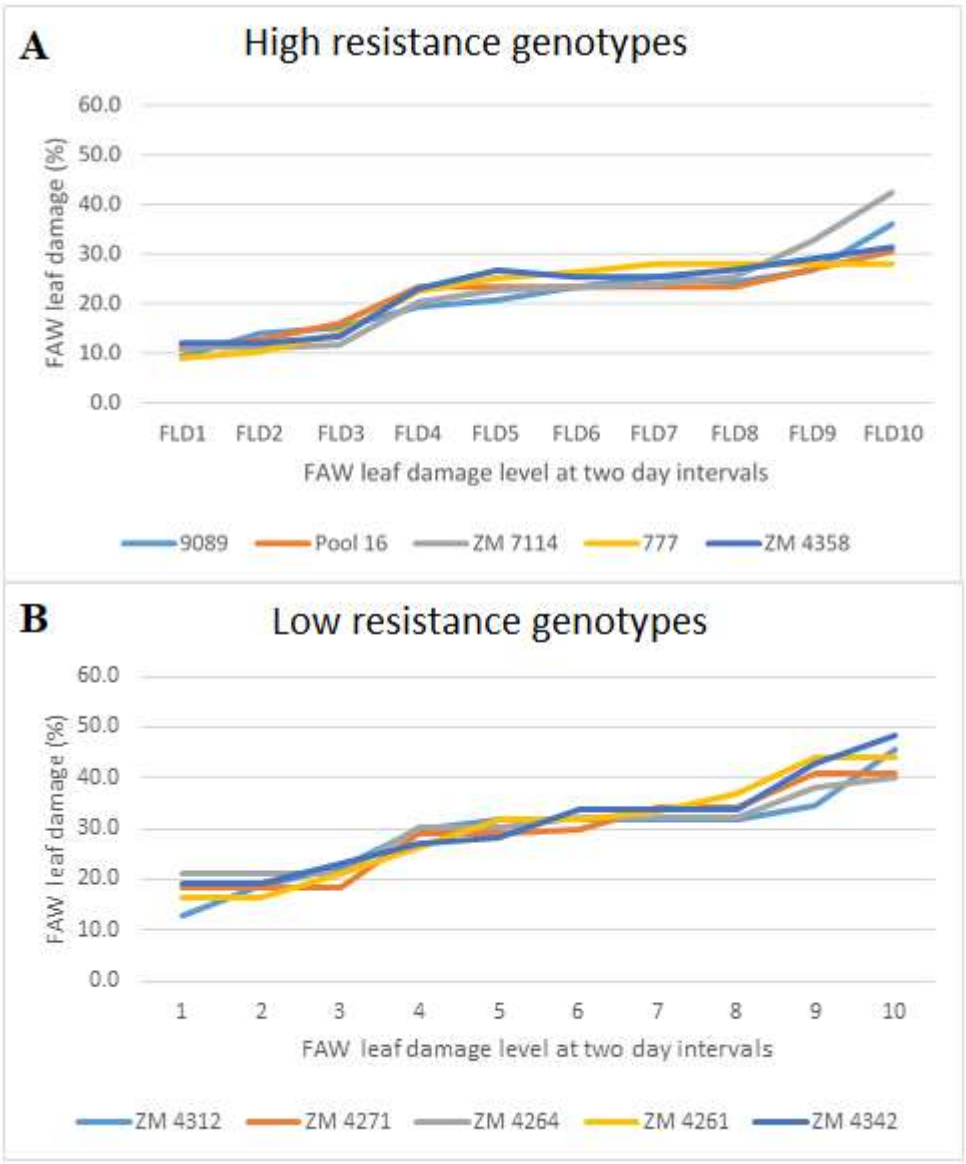

Figure 3.1. Area under pest progress curve for the five most resistant and high resistance (A) (a score of <3) and susceptible and low resistance (B) (a score of >5) genotypes for FAW leaf damage in Set I evaluation

3.3.2 Analysis of variance for agronomic traits and FAW damage parameters

3.3.2.1 Agronomic traits

The genotypes in Set I evaluation exhibited significant variation ($p < 0.001$) for days to flowering, plant height, ear rot, ear aspect, final yield and anthesis-silking-interval ($p< 0.05$) (Table 3.4). Similarly, all agronomic traits varied significantly among the genotypes in Set II evaluation (Table 3.5).

3.3.2.2 FAW parameters

The analysis of variance for the seven non-redundant FLD score-sets in Set I revealed that the genotypes were only significantly different at FLD7, FLD9 and FLD10 (Table 3.4). The differences among genotypes were highly significant ($p< 0.001$) at FLD9, which was scored at 30 days after the first infestation was observed in the field. Highly significant differences ($p< 0.001$) were also obtained for the AUPPC scores of the genotypes. Typical leaf damage levels of the least, moderately and highly damaged plants observed in the evaluations are shown in Figure 3.2. Significant differences ($p< 0.01$) among genotypes were also observed for FCD for Set I genotypes. In the evaluation of Set II genotypes, significant differences ($p<0.05$) occurred for FLD1, FLD2 and FCD. Typical FAW damage observed on maize cobs for FCD during the evaluations are shown in Figure 3.3.

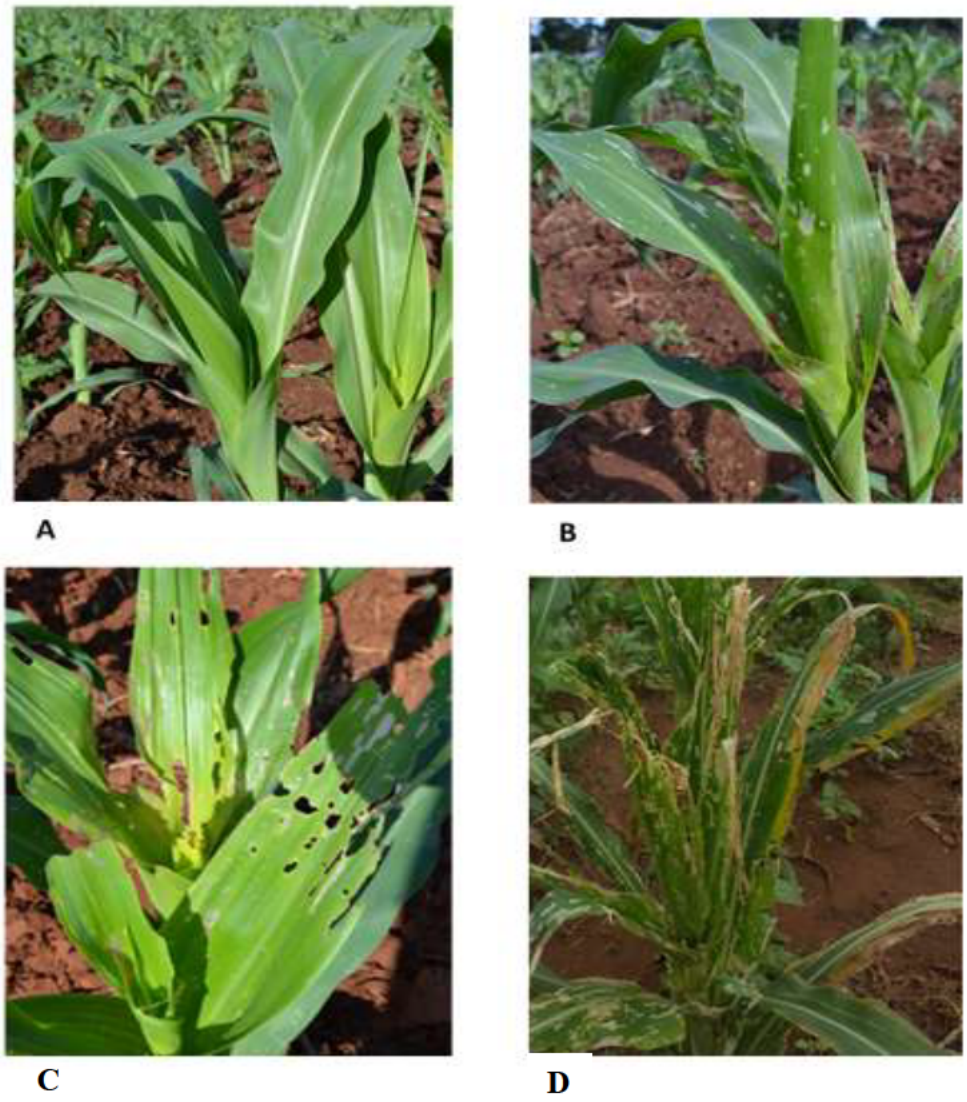

Figure 3.2. Variable FAW reaction types of maize genotypes at mid-whorl growth stage observed during Set I evaluation. Note: A - healthy plant (genotype Pool 16), B - low FAW damage (genotype MM502), C - moderate FAW damage (genotype ZM 7114) and D - severe FAW damage (genotype CZL16026).

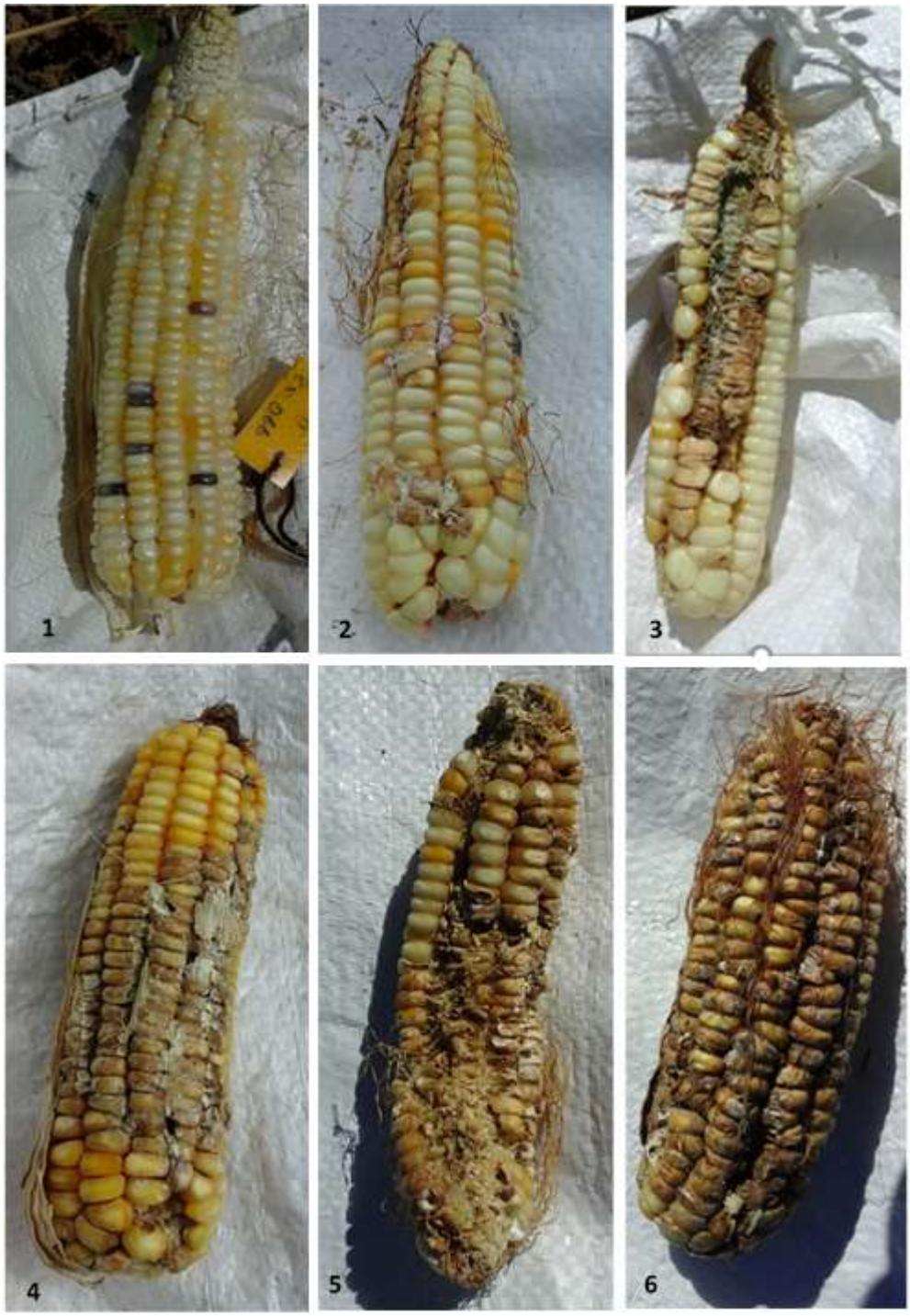

Figure 3.3. Variable FAW damage on maize cobs observed during Set I evaluation. Note: 1- no damage (genotype ZM 4327), 2- mild damage (genotype ZM 4327), 3 - intermediate damage (genotype ZM 4329), 4 - moderate damage (genotype ZM 4234), 5 - Substantial damage (genotype ZM 4234) and 6- severe damage (genotype ZM 4234).

Table 3.4. Analysis of variance and F-statistic tests for agronomic traits and FAW damage when assessing 60 maize genotypes in Set I

		Agronomic traits							FAW parameters								
Source of Variation	Df	DTA	DTS	ASI	PH (cm)	ER (%)	EASP	GY (t ha^{-1})	FLD1 (%)	FLD3 (%)	FLD4 (%)	FLD5 (%)	FLD7 (%)	FLD9 (%)	FLD10 (%)	AUPPC	FCD (%)
Rep	2	28.03	34.06.	0.35	0.27	1665.30	2.17	17.57	17.11	58.65	208.28	40.84	68.74	936.19	720.28	36200	454.80
Rep.Block	27	12.69	13.93	0.21	0.21	247.40	1.42	1.60	49.72	43.82	58.42	57.52	49.70	56.29	115.71	7601	328.30
Genotype	57	34.41***	36.48***	0.38*	0.31***	318.30***	2.37***	8.46***	15.51^{ns}	18.32^{ns}	22.66^{ns}	15.79^{ns}	25.53*	61.62***	67.73**	12571***	289.40**
Residual	89	2.07	2.23	0.20	0.02	130.80	0.85	0.70	18.06	13.51	19.43	19.88	18.03	25.03	39.74	2103	158.50
Total	175	14.55	15.55	0.24	0.15	227.40	1.45	3.54	22.10	20.27	28.66	25.59	25.94	52.28	68.35	6705	230.70

Rep = Replication; * = p < 0.05; ** = p < 0.01; *** = p < 0.001,Df = degrees of freedom, DTA = growing degree day units accumulated to reach to 50 % anthesis; DTS = growing degree units accumulated to 50 % silking; ASI = difference in growing degree day units between DTS and DTA; PH = Plant height; ER = Ear rot; EA = Ear aspect; GY = Grain yield; FLD1 to FLD10 denote FAW field damage score (%) recorded at 14,16, 18, 22 24, 26, 28, 30, and 32 days after the first FAW field infestation, in that order, i.e., FLD1 = The first field damage score taken 22 days after planting, and FLD10 = The tenth field damage score 40 days after planting; AUPPC = Area under pest progress curve; FCD = FAW cob damage

Table 3.5. Analysis of variance and F-statistic tests for maize agronomic traits and FAW damage when assessing 253 maize inbred lines in Set II

		Agronomic traits						FAW parameters		
Source of variation	Df	DTA	DTS	ASI	PH (cm)	WFC (%)	GY (t ha^{-1}) (t/ha)	FLD1 (%)	FLD2 (%)	FCD (%)
Rep	1.00	2.68	7.10	1.09	1.17	955.80	0.01	277.98	46.00	9640.10
Rep.Block	30.00	3.94	4.38	0.24	1.91	702.30	0.06	20.81	93.39	1061.70
Genotype	255.00	107.66***	107.11***	0.99***	9.03***	1361.27***	2.20***	12.77*	62.79***	1471.85***
Residual	206.94	3.92	4.64	0.38	2.09	799.90	0.10	7.49	42.53	799.70
LEE	206.94	4.12	4.84	0.37	2.31	856.15	0.01	10.73	47.93	919.91

Rep = Replication; LEE = lattice effective error used as denominator for the F-test for Genotype; * = p < 0.05; ** = p < 0.01; *** = p < 0.001; Df = degrees of freedom; DTA = growing degree units accumulated to reach to 50 % anthesis; DTS = growing degree day units accumulated to 50 % silking; ASI = difference in growing degree day units between DTS and DTA; PH = Plant height; WFC = Number of well = formed cobs; GY = Grain yield; FLD1 = FAW damage at early-whorl stage; FLD2 = FAW damage at mid whorl stage; FCD = FAW cob damage

3.3.3 Genotype response for agronomic traits and FAW damage

3.3.3.1 Agronomic traits in Set I evaluation

For Set I evaluation, the average DTA and DTS were 836.14 and 859.63 GDDU, respectively (Table 3.6). Genotype Pool 16 was the earliest to attain 50% flowering (687.50 GDDU), while ZM 4327 was the latest (933.50 GDDU) (Appendix 3.2). The average growing degree day units for the period between anthesis and silking for all genotypes was 23.49 GDDU. Plant height ranged from 173 to 251cm. The tallest and shortest genotypes were ZM 4234 (251 cm) and Pool 16 (131 cm). The tallest genotypes in this study were mostly landraces. Ear rot reaction ranged from 7.31 to 57.33% with a mean value of 29.18%. Genotypes ZM 4253 and MM 501 exhibited the highest susceptibility to ear rot and the highest number of cobs damaged by ear rot. Conversely, the genotypes DKC 9089 and ZM 4327 were least susceptible to ear rot and exhibited and the lowest number of cobs damaged by ear rot. Grain yield ranged from 1.05 to 8.24 $ha^{-1}$with a mean of 3.45 t ha^{-1}. PHB 30G19 expressed the highest grain yield at 8.24t ha^{-1}. Among the landraces, ZM 6868 with grain yield of 4.29 t ha^{-1} was the best yielder, while ZM 4253 had the lowest grain yield of 1.05 t ha^{-1}.

3.3.3.2 FAW parameters in Set I evaluation

Leaf damage scores at FLD7, FLD9 and FLD10 were used to rank genotype resistance of FAW larva. Using AUPPC score of individual genotypes, the top ten genotypes with the least AUPPC scores were similar to those ranked using the mean of FLD7, FLD9 and FLD10. Among the top 10 genotypes with the least amount of damage were DKC 8053, Pool 16, DKC 777 and PHB 30G19 (Table 3.6). Most genotypes exhibited relatively high FLD scores (Appendix 3.2). The highest and lowest scores for FAW damage recorded at the mid-whorl stage (FLD9) were 32.00 and 22.67%, respectively. Most of the hybrid checks, including DKC 8053, PHB 30G19, DKC 777 and DKC 9089, were ranked among the top ten genotypes. These included Pool 16, an early maturing open pollinated variety, which was consistently ranked among the top three genotypes for the FLD7, FLD9 and FLD10 ratings. The FCD also varied among the genotypes, ranging from 6.04 to 49.71% with a mean of 24.16% (Table 3.6). The least and highest levels of FAW cob damage were recorded in the genotypes ZMS 638 (6.04%) and ZM 7147 (55.02%), respectively. Nearly 50% of the hybrids evaluated in the study performed well with FCD values below 20%, while the remaining genotypes were comparable to most of the landraces with FAW cob damage values above 20%.

Table 3.6: Mean values for agronomic traits and FAW damage of the top 10 genotypes when assessing 60 maize genotypes in Set I

	Agronomic traits							FAW parameters											
Genotype	DTA	DTS	ASI	PH (cm)	ER (%)	EASP	GY (t/ha)	Genotype	FLD 7 (%)	Genotype	FLD 9 (%)	Genotype	FLD 10 (%)	Genotype	Mean FLD (%)	Genotype	AUPPC	Genotype	FCD (%)
PHB 30G19	788.0	817.5	29.5	218	10.81	1.30	8 24	Pool 16	19.23	DKC 8053	22.67	DKC 8053	22.67	DKC 8053	24.22	Pool 16	361 50	ZMS 638	6.04
SY 5944	840.0	870.0	30.0	208	19.10	1.66	7 98	ZM 7305	23.33	PHB 30G19	26.00	DKC777	25.33	Pool 16	25.54	DKC 9089	383.00	PHB 30G19	6.54
ZMS 638	828.0	850.0	22.0	225	19.91	2.46	7 58	ZM 7114	23.83	DKC 9089	26.67	ZMS 638	29.67	DKC 777	26.89	ZM 7114	395 30	SY 5944	18.50
DKC 777	817.5	840.0	22.5	198	8.84	2.35	8.01	ZM 4310	24.33	Pool 16	26.93	PHB 30G19	30.33	PHB 30G19	27.89	DKC777	396.70	DKC 777	19.10
PAN 7M-83	840.0	850.0	10.0	213	23.63	2.37	7.43	ZM 4253	24.67	DKC 777	27.33	DKC 80-33	30.33	ZM 4358	28.44	DKC 8053	404.00	PAN 7M-83	19.32
DKC 80-33	788.0	817.5	29.5	183	19.62	4.35	4 98	ZMS 606	24.67	ZM 4358	29.00	Pool 16	30.47	DKC 9089	29.33	ZM 4358	404.70	DKC 80-53	19.97
DKC 80-53	788.0	817.5	29.5	177	18.42	1.98	7.00	ZM 7421	24.83	SY 5944	31.00	ZM 4337	31.33	ZM 5021	29.78	ZMS 606	404.80	DKC 80-33	22.62
DKC 9089	817.5	828.0	10.5	16	7.31	1.61	7 38	ZM 4318	25.00	ZM 5021	31.00	ZM 4358	31.33	ZM 6868	30.11	MM 502	411.70	DKC 9089	23.67
ZM 4255	780.0	802.5	22.5	194	48.53	4.96	2 38	ZM 4358	25.00	ZM 6868	31.00	ZM 5021	31.67	ZM 4748	30.44	ZM 4249	413 30	ZM 4255	49.71
ZM 441	767.0	788.0	21.0	184	41.92	4.32	4 11	DKC 9089	25.33	MM 501	32.00	ZM 4748	32.00	ZM 4249	30.67	PHB 30G19	416 30	MM 400	n.a
Mean	**836.14**	**859.63**	**23.49**	**209**	**29.18**	**3.64**	**3.45**	**Mean**	**28.68**		**35.64**		**37.18**		**33.75**		**447 20**		**24.16**
CV (%)	**2.17**	**2.21**	**22.14**	**7.11**	**39.11**	**25.10**	**27.93**	**CV (%)**	**14.73**		**14.01**		**16.87**		**10.34**		**10.26**		**51.64**
EMS	**2.06**	**2.24**	**0 19**	**0.02**	**131 33**	**0.88**	**0 95**	**EMS**	**20.06**		**28.54**		**44.63**		**11.82**		**1577.6**		**164.43**
LSD (0.05)	**2.29**	**2.40**	**0.72**	**0.24**	**18.20**	**1.47**	**1 54**	**LSD**	**7.54**		**9.20**		**11.45**		**5.58**		**74.03**		**20.08**
SE	**1.43**	**1.50**	**0 36**	**0.15**	**11.38**	**0.91**	**0 95**	**SE**	**4.66**		**5.69**		**7.08**		**3.44**		**45.86**		**12.56**
SED	**1.17**	**1.22**	**0.03**	**0.12**	**10.47**	**0.77**	**0 80**	**SED**	**3.81**		**4.64**		**5.78**		**2.81**		**32.43**		**10.11**

CV = Coefficient of variation; EMS = Error mean square; LSD = Least significant difference; SE = Standard error; SED = Standard Error of a difference; n.a = not available; DTA = growing degree day units accumulated to reach to 50 % anthesis; DTS = growing degree day units accumulated to 50 % silking; ASI = difference in growing degree day units between DTS and DTA; PH = Plant height; ER = Ear rot; EA = Ear aspect; GY = Grain yield; FLD7, FLD9 and FLD10 denote FAW field damage score (%) recorded at 26, 30, and 32 days after the first FAW infestations, in that order; FCD = FAW cob damage

3.3.3.3 Agronomic traits in Set II evaluation

There was a wide variation for agronomic traits among Set II genotypes. The mean DTA and DTS for all the genotypes was 739.05 and 763.92, respectively (Table 3.7). Compared to other variables, the ASI showed less variation among genotypes, with a mean of 24.88 GDDU. Cob-filling (WFC) percent ranged from 0 to 98.81%, while the average FNP per entry was 5.00. Grain yield ranged from 0.02 to 6.53 t ha^{-1}. Inbred line CML312-2 had the highest grain yield (6.53t ha^{-1}), which exceeded that of the highest yielding hybrid check DKC 777 (4.82t ha^{-1}). Genotype CML486-216 exhibited considerably higher GY (6.07 t ha^{-1}).The genotypes that were most affected by FAW in terms of leaf and cob damage included VL054530, CZL16026 and CZL055011 (Appendix 3.3).

3.3.3.4 FAW parameters in Set II evaluation

The FAW leaf damage progressively increased from FLD1 to FLD2 among the 253 inbred lines evaluated in Set II (Table 3.7). The mean FAW damage was 9.87% at FLD1 and 25.45% at FLD2. The highest and lowest mid-whorl stage FAW leaf-feeding damage (FLD2) was 48.13 and 8.72%, respectively. At FLD2, the genotype VL05614 was the least affected by the FAW with an FLD2 score of 8.70%, while the FLD increased to 48.31% in the genotype CZL055011. The mean FLD (averaged across FLD1 and FLD2) and FCD ranged from 8.87% to 28.34% and 5.36% to 100.00%, respectively. The mean FLD and FCD averaged across all genotypes were 17.65% and 62.81%, and genotypes CML304-B and CML442 had the lowest values, respectively.

Table 3.7. Means for agronomic traits and FAW damage recorded for 253 maize genotypes in Set II

Genotype	DTA	DTS	ASI	FNP	WFC (%)	GY (t ha^{-1})	FLD1 (%)	FLD2 (%)	FLD Mean (%)*	FCD (%)
Top 10 genotypes (low FAW damage)										
CML304-B	888.0	918.5	30.5	2	50.23	0.10	6.25	11.50	8.87	14.42
CZL15231	625.5	658.5	33.0	1	67.45	0.13	3.74	14.96	9.33	16.03
VL05614	n.a	n.a	n.a	3	49.47	0.34	10.85	8.72	9.75	49.60
CML-486-216	645.5	658.5	13.0	9	43.51	6.07	5.29	15.69	10.45	51.64
CML442	n.a	n.a	n.a	3	50.02	0.18	4.37	18.99	10.82	5.36
CZL16090	918.5	943.0	24.5	1	25.41	0.45	5.29	17.02	11.17	11.41
TL141998	589.0	613.5	24.5	2	74.86	0.35	5.16	17.28	11.23	74.66
CML539	574.0	613.5	39.5	5	62.16	0.78	5.81	17.06	11.41	74.18
TL115627	740.5	768.0	12.0	5	79.44	0.81	11.95	11.89	11.65	46.38
TL1512847	600.5	645.5	45.0	8	52.53	1.12	7.14	17.01	12.06	45.11
Bottom five genotypes (high FAW damage)										
CML444-B	873.5	888.0	14.5	4	10.00	0.69	10.2	40.62	25.4	47.58
VL054530	862.5	873.5	11.0	2	98.81	0.11	8.85	42.46	25.62	100
CZL16026	n.a	n.a	n.a	7	50.56	0.29	10.12	44.63	27.42	50.72
CZL055011	625.5	687.0	20.0	5	32.87	0.67	9.04	48.13	28.34	56.15
TL122165	n.a	n.a	n.a	4	0.00	0.25	n.a	n.a	n.a	7.20
Mean	**739.05**	**763.92**	**24.88**	**5**	**54.4**	**0.75**	**9.87**	**25.45**	**17.65**	**62.81**
CV (%)	**2.55**	**2.76**	**34.81**	**29.17**	**50.53**	**40.25**	**31.04**	**25.55**	**21.09**	**44.76**
LSD (0.05)	**3.48**	**3.86**	**1.70**	**2.95**	**57.45**	**0.64**	**6.14**	**12.98**	**7.43**	**57.75**
SE	**1.78**	**1.97**	**0.61**	**1.43**	**28.03**	**0.38**	**3.07**	**6.50**	**3.72**	**28.17**
SED	**1.81**	**1.99**	**0.60**	**1.49**	**29.11**	**0.31**	**3.22**	**6.81**	**3.90**	**29.96**

CV = Coefficient of variation; EMS = Error mean square; LSD = Least significant difference; SE = Standard error; SED = Standard Error of a difference; n.a = not available; DTA = growing degree day units accumulated to reach to 50 % anthesis; DTS = growing degree day units accumulated to 50 % silking; ASI = difference in growing degree day units between DTS and DTA; FNP = Final number of plants; WFC = Well-filled cobs; GY = Grain yield; FLD1 = Early-whorl stage FAW damage; FLD2 = Mid whorl stage FAW damage; Mean FLD = average of FLD1 and FLD2; FCD = FAW cob damage.*the genotypes were ranked according to mean FLD

3.3.4 Principal component (PC) analysis

Principal component (PC) analysis for FAW resistance and maize agronomic traits shows that the first and second principal components (PC1 and PC2) accounted for 34.00 and 25.50 % of the total variation, respectively in Set I. Nine traits contributed highly to the total variation accounted for by PC1 and PC2in Set I genotypes (Table 3.8). Traits FLD2, mean FLD, GY, EA, DTA and DTS contributed the highest variation in PC1, while FCD, DTS, DTA and PH had high PC2 loadings. FLD 9 and mean FLD had high positive loadings, while GY had high negative loadings in PC1 with values greater than 0.70 each. For PC2, the highest positive loadings were contributed by DTA and DTS with values above 0.70 each and the least was by FCD (-0.81). FCD and ASI contributed the weakest positive impact on PC1 and PC2,

respectively. Conversely, ER and FLD2 had the weakest loadings on PC1 and PC2, which were both less than -0.20.

In Set II, PC1 and PC2 accounted for 23.00 and 18.90% of the total variation, respectively. FLD1, FLD2, mean FLD, DTA, DTS and ASI had positive loadings correlated with PC1. FLD1, FCD and ASI had positive loadings of less than 0.20, while the rest loaded more than 0.65 each on PC1. In addition, DTA, DTS, ASI, FNP and GY contributed positively to PC2. The phenological traits, DTA and DTS had the strongest contribution of 0.70 each to the PC2, while FNP and GY contributed 0.43 each. FCD had weak positive impact on PC1 (Table 3.8). In both Set I and II, FAW leaf damage contributed more to the observed total variation in the first two PCs than FCD.

Table 3.8. Agronomic and FAW parameters and their contributions to principal component1 (PC1) and principal component 2 (PC2) in Set I and Set II genotypes.

Set I			Set II		
Traits	PC1	PC2	Traits	PC1	PC2
Eigen values	4.09	3.06	Eigen values	2.33	1.88
Proportion of variation (%)	34.11	25.53	Proportion of variation (%)	23.30	18.80
Cumulative variation (%)	34.11	56.64	Cumulative variation (%)	23.30	42.10
FLD2	0.37	-0.04	FLD1	0.19	-0.15
FLD 9	0.77	-0.27	FLD2	0.75	-0.43
Mean FLD	0.76	-0.23	Mean FLD	0.76	-0.45
DTA	0.63	0.71	DTA	0.67	0.70
DTS	0.64	0.71	DTS	0.68	0.71
ASI	0.34	0.33	ASI	0.12	0.07
PH	0.53	0.69	FNP	-0.22	0.43
ER	-0.15	-0.26	WFC	-0.23	-0.03
EA	0.64	-0.47	GY	-0.34	0.43
GY	-0.76	0.44	FCD	0.10	-0.34
FCD	0.05	-0.81			

DTA = growing degree day units accumulated to reach to 50 % anthesis; DTS = growing degree day units accumulated to 50 % silking; ASI = difference in growing degree day units between DTS and DTA;PH = Plant height; ER = Ear rot; EA = Ear aspect; FNP = Final number of plants; WFC = Well-filled cobs; GY = Grain yield; FLD1 = Early-whorl stage FAW damage in Set II; FLD2 = mid-whorl stage FAW damage in Set II;FLD2 = early whorl-stage FAW damage in Set I; FLD9 = mid whorl stage FAW damage in Set I; Mean FLD = average FAW damage; FCD = FAW cob damage.

The relationship between measured traits and the genotypes in the evaluations of the two sets of genotypes is depicted by the principal component biplots (Figure 3.4). Unidirectional line vectors with smaller angles in-between represent closely correlated traits with respect to the capacity to differentiate the genotypes. Genotypes with high performance for a given trait are located close to the line vector representing a trait, and close to or on the vertex of the biplot.

In Set I, the majority of the genotypes tended towards the first quadrant with low scores for FCD and ER. However, some genotypes such as E28 (ZM 4253), E41 (ZM 4251) and E49 (ZM 4256) exhibited very low GY values although they scored higher on ear aspect. E9 (ZM 6868) and E10 (PHB 30G19) excelled in GY and FCD, while E1 (DKC 8053) and E2 (DKC 777) excelled in the FLD1, FLD9 and mean FLD (Figure 3.4A). In Set II, the genotypes were generally clustered in the second and third quadrants with almost similar variation explained by the two PCs (Figure 3.4B). Genotypes such as E1 (CML304-B), E6 (CZL16090) and E17 (EBL173778) excelled in FCD, FLD1, and FNP. In terms of the combination of DTA and WFC, E3 (VL05614), located midway between the line vectors for the two traits was the winning genotype with relatively less days to 50% anthesis and high cob filling percent. (Figure 3.4B).

In both Sets I and II, the FAW related traits FLD2, FLD9, mean FLD and FCD were positively correlated to each other (Table 3.9). The FLD2 showed significant associations with FLD9 ($r=0.43$, $p<0.001$) and mean FLD ($r=0.68$, $p<0.001$). The association between mean FLD and FDL9 was also significant ($r=0.94$, $p<0.001$). FLD2 had no significant association with any other agronomic traits though it showed a tendency to be negatively associated with GY. Conversely, FLD9 and mean FLD exhibited significant associations with some agronomic traits. FLD9 had a positive and significant association with EA ($r=0.42$, $p<0.001$), while it had a negative and significant association with GY ($r=-0.41$, $p<0.001$). Mean FLD exhibited similar associations with EA, and GY to FLD9. FLD9 had the highest negative correlation with GY. DTA and DTS exhibited significant associations with FCD ($r=0.50$, $p<0.001$), ASI ($r=0.30$, $p<0.001$) and ER ($r=0.31$, $p<0.01$). High yielding genotypes were more susceptible to ER as exhibited by a positive association between ER and GY ($r=0.27$, $p<0.05$).

In Set II, FLD1 had non-significant association with yield ($r = 0.06$). The remaining FAW related traits FLD2, mean FLD, FCD were all negatively correlated with yield. The only significant association between pairs of agronomic traits was between DTS and WFC ($r=-0.13$, $p<0.05$) (Table 3.9).

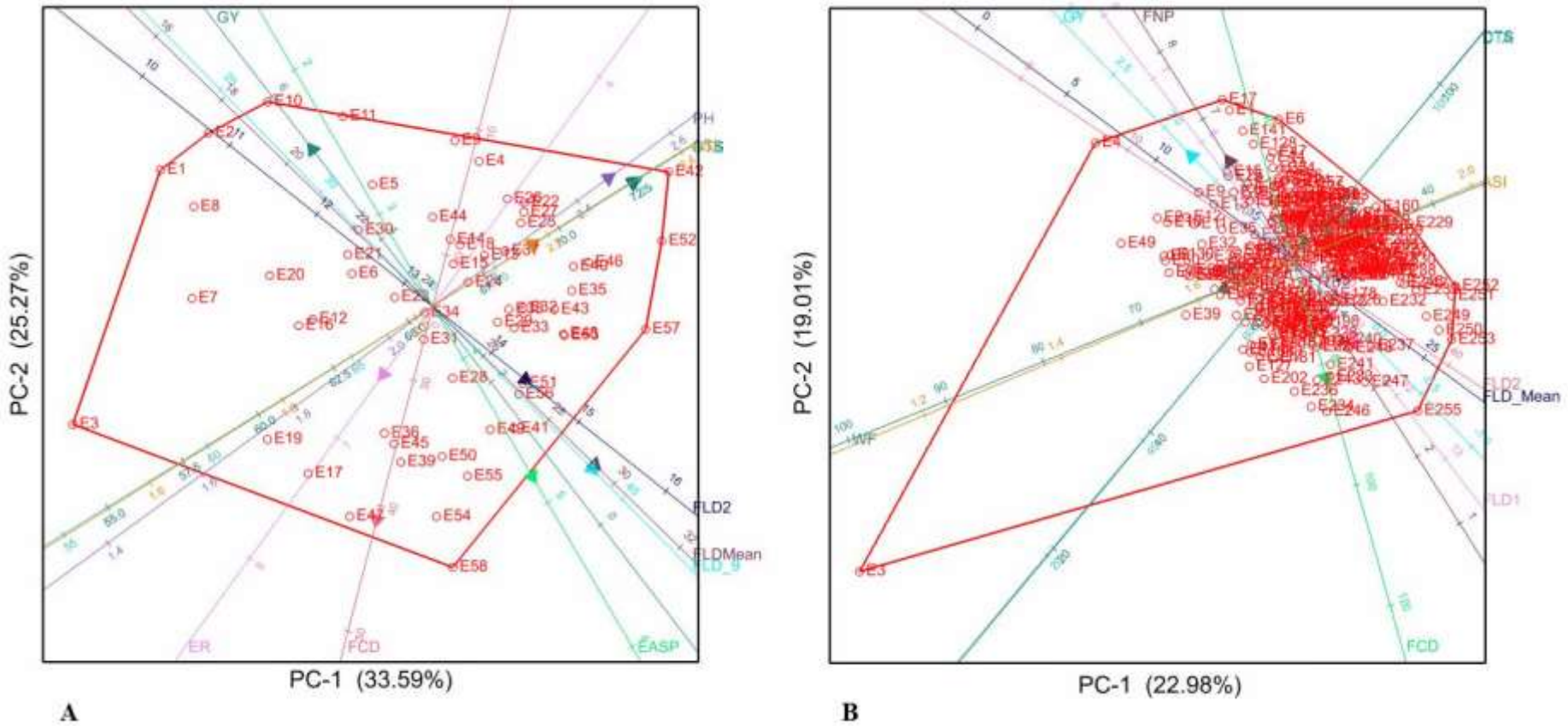

Figure 3.4. Principal component biplots with the best genotypes for traits measured in Set I (A) and Set II (B) genotypes.

DTA = growing degree day units accumulated to reach to 50 % anthesis; DTS = growing degree day units accumulated to 50 % silking; ASI = difference in growing degree day units between DTS and DTA; PH = Plant height; ER- Ear rot; EASP = Ear aspect; FNP = Final number of plants; WF = Well-filled cobs; GY = Grain yield; FLD1 = Early = whorl stage FAW damage in Set II; FLD2 = mid-whorl stage FAW damage in Set II; FLD2 = early-whorl stage FAW damage in Set I; FLD9 = mid-whorl stage FAW damage in Set I; Mean FLD = average FAW damage; FCD = FAW cob damage. Note: The codes of genotypes are provided in Appendices 3.4 and 3.5.

Table 3.9: Pearson pairwise correlation coefficients showing pairwise associations among FAW induced damage and agronomic traits measured on 60 maize genotypes in Set I and 253 genotypes in Set II

Set I evaluation

Trait	FLD2	FLD9	Mean FLD	DTA	DTS	ASI	PH	ER	EA	GY	FCD
FLD2[I]	1.00										
FLD9	0.43***	1.00									
Mean FLD	0.68***	0.94***	1.00								
DTA	0.15	0.13	0.19	1.00							
DTS	0.15	0.14	0.20	1.00	1.00						
ASI	-0.13	-0.02	-0.01	0.32**	0 37**	1.00					
PH	0.15	0.08	0.15	0.78	0.77	0.41***	1.00				
ER	-0.20	0.06	-0.01	-0.31**	-0.31**	0.05	-0.17	1.00			
EA	0.04	0.42***	0.35**	0.06	0.07	0.09	-0.04	0.08	1.00		
GY	-0.17	-0.41***	-0.41***	-0.07	-0.08	0.01	0.02	0.27*	-0.49***	1.00	
FCD	0.02	0.15	0.16	-0.49***	-0.50***	-0.24	-0.41***	0.20	0.36**	-0.42***	1.00

Set II evaluation

Trait	FLD1	FLD2	Mean FLD	DTA	DTS	ASI	FNP	WFC	GY	FCD
FLD1	1.00									
FLD2	0.04	1.00								
Mean FLD	0.41	0.01	1.00							
DTA	0.01	0.06	0.05	1.00						
ASI	0.00	0.06	0.06	0.99	1.00					
DTS	0.07	0.00	0.04	0.07	0.03	1.00				
FNP	0.19 **	0.12*	0.03	0.01	0.00	0.07	1.00			
WFC	0.13*	0.05	0.00	0.12	0.13*	0.08	0.11	1.00		
GY	0.06	0.23***	0.18**	0.00	0.00	0.04	0.64	0.35	1.00	
FCD	0.01	0.12	0.10	0.10	0.10	0.03	0.08	0.08	0.15*	1.00

* = p < 0.05; ** = p < 0.01; *** = p < 0.001; DTA = growing degree day units accumulated to reach to 50 % anthesis; DTS = growing degree day units accumulated to 50 % silking; ASI = difference in growing degree day units between DTS and DTA; PH = Plant height; ER = Ear rot; EA= Ear aspect; FNP = Final number of plants; WFC = Number of well-filled cobs; GY = Grain yield; FLD2[I] = Early-whorl stage FAW leaf damage in Set I; FLD9 = mid-whorl stage FAW leaf damage in Set I; FLD1 = early- whorl stage FAW leaf damage in Set II; FLD2 = mid whorl stage FAW leaf damage in Set II; Mean FLD = Average FAW leaf damage; FCD = FAW cob damage

3.4 Discussion

This study examined two diverse sets of maize germplasm to explore their genetic variation as sources of resistance to FAW. The study site had an active natural FAW population during the maize production season and off-season resulting in severe infestations and damage to maize. Although this enabled clear discrimination among the test genotypes based on their reaction to FAW infestation, these results need further confirmation involving multiple season and site evaluations. Within 14 days of the trials being planted, FAW started feeding on the emerging maize plants resulting in severe FAW damage. Multiple subsequent scoring using the Set I genotypes showed little change in FAW leaf-damage progress between 16 and 18 days (FLD2 and FLD3), and 24 and 28 days (FLD6 and FLD8) after the first signs of FAW infestation. The small changes in leaf damage measured at two-day intervals suggested that a slightly extended interval between scoring sessions could be sufficient to detect genotype differences among successive sampling dates. Therefore, the scoring interval was adjusted from two to six days between scoring sessions for the second trial, and this interval was validated during the evaluation of Set II genotypes, which commenced 16 days after the first FAW infestation.

Evaluation of the Set II genotypes indicated that the best time to compare maize genotypes based on FAW leaf-damage scores was at the vegetative growth stage 4-5 (22-28 days after the onset of infestation) corresponding to FLD2 in the Set II evaluation). This is in agreement with Morrill and Greene (1974), who reported that maize is less sensitive to late-whorl FAW infestations than early- and mid-whorl stage infestations. However, based on the observations made during the present study, there are indications that FAW leaf-damage scores should be recorded at least four times during the vegetative growth stage, ideally between 16-30 days after the initial infestation to reduce the confounding effects of temperature, seasonality and the crop's developmental stage. These factors affect the length of the FAW life cycle and, therefore, the number of FAW generations per season, leading to variation in pest pressure. Multiple recordings of FLD levels enabled a more accurate assessment of FAW leaf-injury across the critical early- and mid-whorl stages of growth.

Leaf damage at the mid-whorl stage (approximately FLD9 and FLD2 for Set I and II evaluations, respectively) was higher in the Set II evaluation (48.13%) compared to the Set I evaluation (32%). Thus, the inbred lines of Set II exhibited higher susceptibility to FAW damage than the landraces in Set I. Inbred lines have slower growth rates due to inbreeding depression (Ibraheem and El-Ghareeb, 2019) enabling the FAW larvae to feed for longer on the same whorl tissue before the plant grows past the whorl stage, which is the preferred feeding

stage for FAW larvae. In contrast, landrace, OPVs and hybrids have higher vigour when compared with inbred lines (Fu et al., 2014). The landraces, OPVs and hybrids grow faster and take a relative shorter period to pass through the preferable feeding stage for FAW larvae (Azeez et al., 2018; Prasanna et al., 2018). The prolonged feeding by the FAW caused pronounced leaf damage symptoms on the inbred lines in Set II evaluation resulting in relatively higher leaf damage ratings for Set II compared to Set I evaluation. Similarly, crops with poor growth vigour due to nutrient deficiencies or other factors can show significant leaf damage, which contributed to a general overestimation of FAW impact on maize yield in SSA (Baudron et al., 2019). Thus, recommended agronomic practices must be followed to reduce the number other factors that could exacerbate the effect of FAW damage on maize. The high levels of FAW damage in the inbred lines in Set II evaluation could be attributable to the trial being conducted in the off-season, when there was minimal cropping activities in the environment where the trials were conducted. This may have led to increased FAW pressure in this trial, where maize was the main host for FAW in the surrounding environment. However, the comparable final damage levels between the tested inbred lines in Set II and the landraces in Set I suggest that there is need for further studies to investigate the mechanisms of FAW leaf damage and possible compensation by the maize genotypes. Thus, the nature of the tested germplasm, the season in which evaluations are made, and the crop growth stage are important factors to consider during scoring for FAW because they are likely to influence the intensity of infestation and the severity of damage.

The highest FAW leaf damage occurred at the mid-whorl stage, during both the main season (43.67% leaf damage) and off-season (48.13 % leaf damage) evaluations. This suggests that this stage may be appropriate for germplasm evaluation for resistance to FAW, at least based on the conditions of these two trials. FAW moths lay their eggs on maize leaves (Assefa et al., 2019). Mechanical control such as physical removal and crushing of larvae and egg masses, and chemical control methods would be more effective during the early instar stage before the young larvae move into the leaf whorl. Cultural control methods such as early planting and intercropping are recommended to reduce potential FAW damage (Kansiime et al., 2019; Harrison et al., 2019). The seedling stage of maize is the most vulnerable to FAW attack. Abate et al. (2000) and Kumela et al. (2018) reported that smallholder farmers in Africa cannot afford chemical pesticides and resort to using mechanical methods (e.g. handpicking of larvae and application of sand/or ash) to control FAW. However, mechanical methods will only be effective before the larvae become more concealed within the leaf whorl. Chemical control

measures should be used before the larvae is concealed in the leaf whorl (Day et al., 2017). Harnessing mid-whorl stage resistance appears promising for reducing the number of chemical spray applications for FAW management in maize. The lower levels of damage that occurred during the main season was probably due to the presence of other feed sources, including several summer host crops. Although the pest has many other hosts, maize is the preferred host (FAO, 2018). This has implications for agronomic practices such as fallowing and crop rotation in order to deprive the FAW of other food sources, as a way to reduce its population growth. Baudron et al. (2019) conducted a study in the eastern Zimbabwe, which experiences similar climatic conditions to the current study site. The authors reported that the level of damage caused by FAW in maize varieties ranged between 32-48%, concurring with the present findings. The damage may be variable across test sites due to differences in genotypes, and the local environment and their interaction. Nonetheless, the reported damages were significant, showing the importance of the pest in Zambia.

In Set II evaluation, low levels of FAW feeding occurred on 18% of the genotypes, whereas 70% of the genotypes suffered moderate levels of feeding. The genotypes with signs of low levels of FAW feeding included inbred lines and all three commercial checks in Set II. Currently, there are no maize cultivars adapted to SSA growing conditions that have been confirmed to have high levels of resistance or tolerance to FAW (Prasanna et al., 2018). Hence the genotypes that were least or moderately damaged by FAW could potentially harbour some FAW resistance or tolerance genes for use in developing FAW resistant cultivars suitable for SSA agro-ecologies. A total of 30 genotypes identified from Set I and II evaluations, consisting of five Zambian genotypes and 25 CIMMYT-developed inbred lines, were selected from the genotypes that exhibited low or moderate feeding damage by FAW (Appendix 3.6). Pool 16, which is an extra early maturing improved OPV, originally developed by CIMMYT (Masole and Gumbo, 1994), was among the local genotypes selected as a breeding parent. Pool 16 and ZM 7114 were among the genotypes with low AUPPC values (Table 3.3).

Genotypes with the CML (CIMMYT maize line) background have been recommended previously for their adaptation to southern African conditions. Most CML genotypes attain yields above 3.0 t ha^{-1} (Wu et al., 2016) and four were among the inbred lines selected for genetic advancement. The four selected CMLs (CML545-B, CML539 CML548-B and CML491) are early, early-intermediate, intermediate and late maturity lines, respectively. These are reportedly excellent combiners with the popular inbred lines CML395, CML441 and CML312 that are locally adapted and are already widely used in southern Africa (Masuka et

al., 2017). CML491 is a QPM line and has been scored as 6.0 for FAW resistance on the Davis et al. (1992) scale, while CML539 is partially resistant to maize streak virus (MSV) (CIMMYT, 2019). CML545-B and CML548-B possess high levels of tolerance to common mid-altitude foliar diseases of maize such as grey leaf spot (*Cercospora zeae-maydis* Tehonand Daniels), northern corn leaf blight (*Setosphaeria turcica* Leonard and Suggs) and common leaf rust (*Puccinia sorghi* Schwein.). These genotypes were recommended for use as male and female breeding parents, respectively (CIMMYT, 2019). In addition to low levels of FAW leaf damage, the inbred lines CML545-B, TL173, TL1316 and TL1359 were considered for useful agronomic traits and selected for breeding. In particular, these genotypes displayed short anthesis-silking-interval, a trait often associated with drought tolerance. Regarding FAW leaf damage, selection of genotypes from low, moderate and high-resistance groups reportedly increased the chances of accumulating horizontal resistance genes (Kim and Brewbaker, 1977; Vanderplank, 1978). The key factors used as the basis of selection of genotypes were: genetic background of the tested germplasm such as CIMMYT maize lines (CMLs), CIMMYT Zimbabwe Lines (CZLs), and CIMMYT Kenya Hybrids (CKH), response to drought stress, husk cover, level of FAW leaf damage, level of FAW cob damage, QPM status and recovery from FAW feeding damage. This will facilitate the development of new and complimentary lines with multiple traits that are important to farmers and the market. Acknowledging other farmer-preferred traits during the early breeding stages enhances the chances of adoption of the cultivars after release (Shimelis, 2017).

The first two PCs accounted for 59.6% of the total variation in the evaluation of Set I genotypes indicating that genotypes should be selected for improvement based on their performance in traits with high PC1 and PC2 loadings (Table 3.8). The four traits that had the highest loadings on the two PCs were mean FLD, FLD2, DTA and DTS. These should, therefore, be included in FAW resistance breeding. For Set II evaluation, the first two PCs accounted for 41.9% of the variation, showing that there is need to consider other traits, with substantial loadings on subsequent PCs. The high contribution of the FLD2 trait to PCs showed that early FAW damage was critical when evaluating genotypes to identify promising ones. Buntin (1986) observed that infestation by FAW at early whorl stages was likely to cause more damage than infestation at later whorl stages. Thus, genotypes that exhibited low levels of FAW damage during early stages could potentially harbour FAW resistance. These genotypes included CML539, CML444-B, CML491, CML548-B and CZL15231. Plant height was not among the major contributors to PC1 and PC2 in this study. However, considering that foliar damage

impedes plant growth including plant height, it is recommended that future evaluations make direct comparisons of plant height between FAW infested and non-infested genotypes to accurately determine the relationship among these traits and grain yield.

Principal biplot analysis further revealed genotypes that excelled in specific traits including Pool 16, PHB 30G19, DKC 777 from Set I, and CML304-B, CML304-B, CZL16090 and EBL173778 from Set II, in terms of specific traits. However, not all of the best genotypes for specific traits were considered for further advancement because some of them either lacked important farmer-preferred traits or were poorly adapted to the target conditions. For instance, E17 (EBL173778) had low levels of FAW cob damage but had poor yield potential.

The negative correlation between FAW leaf damage, FAW cob damage and grain yield (Table 3.9) suggested the possibility of identifying high yielding genotypes by considering genotypes with low scores for FAW-related traits. Grain yield exhibited negative correlations with FAW leaf damage and cob damage corroborating previous reports (Hruska and Gould, 1997; Lima et al., 2010; Kumela, 2018). FLD9 had the highest negative correlation (Table 3.9) with grain yield implying that the highest significant yield loss occurs if the pest is not controlled within 30 days after infestation. In addition to the crop growth stage, pesticide application for FAW management is most likely effective when applied to younger FAW larvae, typically during instars 1-2, because of their positive phototropism, which keeps them on the leaf surface. Early pest control is essential to prevent severe yield losses. Although any damage to the leaf can potentially reduce grain yield, leaf damage alone cannot be used as a primary predictor of yield loss in maize (Baudron et al., 2019) because the pest must destroy the entire leaf whorl to cause a significant reduction in grain yield (Lima et al., 2010). There is a need to further investigate the relationship between FAW leaf damage and maize grain yield loss under natural levels of FAW infestation and across sites to establish more accurate prediction models. Despite the ability of maize genotypes to tolerate moderate to large levels of defoliation before significant yield loss occurs, direct damage to yield-determining organs of maize plants results in substantial yield losses (Buntin, 1986; Prasanna et al., 2017). On the other hand, FAW cob damage represents a more direct indicator of the impact of FAW on grain yield. However, cob damage cannot be used as a predictor because it can only be measured at the end of the growing season, when it is too late to implement mitigation strategies.

3.5 Conclusion

The current study examined two sets of maize genotypes for FAW resistance and for desirable agronomic traits. Field studies were conducted under natural FAW infestation in Zambia. Leaf and cob damages are the traits most strongly associated with FAW damage in maize, which can be used as selection criteria for resistance to FAW. The best time to score for FAW resistance in our trials based on leaf damage levels was at vegetative growth stages 4 to 5, corresponding to between 22 and 28 days after the first sign of field infestation by FAW. However, this window period is dependent on other factors including the onset of FAW infestations in maize. FAW cob damage, assessed at harvest provides a more direct selection criterion for identifying maize genotypes that incur less FAW damage. Thirty promising genotypes were identified as a basis for developing a FAW maize breeding population. These included both inbred lines and landraces that showed moderate to high levels of resistance to FAW feeding. The study selected unique genotypes such as Pool 16, ZM7114, CZL1310c, CML444-B, CZL15220 and TL1512847 for their farmer-preferred traits and better agronomic values. The new selections are useful to breed and deploy maize cultivars adapted to Zambia or similar agro-ecologies in SSA.

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3.7 Appendices

Appendix 3.7.1. Leaf damage scales used in the present study to assess Fall Armyworm infestation level in maize

Class	Leaf damage scales		Description
	Present study (%)	Davis et al (1989)	
A	0-10	0-1	● Plants with no damage, windowing signs on leaves, up to five pinholes per leaf
B	11-20	2-3	● Up to eight pinholes per leaf ● Up to five clearly visible shot holes per leaf ● One to two elongated lesions per leaf ● Leaf whorls of one to three plants eaten up
C	21-30	4-5	● > 10 pinholes ● Up to 10 clearly visible shot holes ● 3-5 elongated lesions per leaf ● Leaf whorls of up to five plants eaten up
D	31-40	6-7	● Up to 10 clearly visible shot holes per leaf ● Up to five elongated lesions ● Up to three clearly visible portions eaten away per leaf ● One to two dying areas on a leaf ● Leaf whorls of more than five plants eaten up
E	41-50	8-9	● More than eight elongated lesions per leaf ● More than five portions visibly eaten away ● More than two dying portions on leaf ● Leaf whorl almost entirely eaten (away dead heart with signs of new shoot)
F	>50	10	● Dead heart with no signs of new shoot ● Dying or dead plant ● Severely damaged plant

Note: unable to assign genotypes into discrete class above the 50% leaf damage due to the severity of infestation

Appendix 3.7.2: Mean values of 60 maize genotypes for agronomic traits and fall armyworm damage parameters in Set I evaluation

Agronomic traits								FAW parameters											
Genotype	DTA	DTS	ASI	PH	ER	EASP	GY	Genotype	FLD7 (%)	Genotype	FLD9 (%)	Genotype	FLD10 (%)	Genotype	Mean FLD (%)	Genotype	AUPPC	Genotype	FCD
Top 20 genotypes																			
PHB 30G19	788 00	817 50	29 50	218	10 81	1 30	8 24	Pool 16	19 23	DKC 8053	22 67	DKC 8053	22 67	DKC 8053	24 22	Pool 16	361 50	PHB 30G19	6 54
SY 5944	840 00	870 00	30 00	208	19 10	1 66	7 98	ZM 7305	23 33	PHB 30G19	26 00	DKC777	25 33	Pool 16	25 54	DKC 9089	383 00	SY 5944	18 50
ZMS 638	828 00	850 00	22 00	225	19 91	2 46	7 58	ZM 7114	23 83	DKC 9089	26 67	ZMS 638	29 67	DKC 777	26 89	ZM 7114	395 30	ZMS 638	6 04
DKC 777	817 50	840 00	22 50	198	8 84	2 35	8 01	ZM 4310	24 33	Pool 16	26 93	PHB 30G19	30 33	PHB 30G19	27 89	DKC 777	396 70	DKC 777	19 10
PAN 7M-83	840 00	850 00	10 00	213	23 63	2 37	7 43	ZM 4253	24 67	DKC 777	27 33	DKC 80-33	30 33	ZM 4358	28 44	DKC 80-53	404 00	PAN 7M-83	19 32
DKC 80-33	788 00	817 50	29 50	183	19 62	4 35	4 98	ZMS 606	24 67	ZM 4358	29 00	Pool 16	30 47	DKC 9089	29 33	ZM 4358	404 70	DKC 80-33	22 62
DKC 80-53	788 00	817 50	29 50	177	18 42	1 98	7 00	ZM 7421	24 83	SY 5944	31 00	ZM 4337	31 33	ZM 5021	29 78	ZMS 606	404 80	DKC 80-53	19 97
DKC 9089	817 50	828 00	10 50	160	7 31	1 61	7 38	ZM 4318	25 00	ZM 5021	31 00	ZM 4358	31 33	ZM 6868	30 11	MM 502	411 70	DKC 9089	23 67
MM 400	n a	n a	n a	n a	n a	n a	n a	ZM 4358	25 00	ZM 6868	31 00	ZM 5021	31 67	ZM 4748	30 44	ZM 4249	413 30	MM 400	n a
MM 4255	780 00	802 50	22 50	194	48 53	4 96	2 38	DKC 9089	25 33	MM 501	32 00	ZM 4748	32 00	ZM 4249	30 67	PHB 30G19	416 30	ZM 4255	49 71
MM 441	767 00	788 00	21 00	184	41 92	4 32	4 11	ZM 4258	25 40	ZM 4318	32 33	ZM 6868	32 00	ZM 7305	30 89	MM 501	416 30	MM 441	40 82
MM 501	788 00	817 50	29 50	180	49 19	2 63	6 25	ZM 7147	26 00	ZM 4249	32 67	7M-83	32 67	ZM 4318	31 00	ZM 4253	416 30	MM 501	30 19
MM 502	802 50	828 00	25 50	206	20 75	3 36	5 71	ZM 4237	26 17	ZM 7114	32 67	ZM 4249	32 67	DKC 8033	31 22	ZM 7421	416 70	MM 502	12 48
Pool 16	687 50	714 00	26 50	131	14 21	3 99	1 10	ZM 4323	26 33	ZM 4234	33 00	ZM 4327	33 67	ZM 4253	31 28	ZM 4236	417 00	Pool 16	25 62
ZM 3676	860 00	885 00	25 00	230	20 66	3 69	2 25	ZM 4329	26 33	ZM 4307	33 00	ZM 4324	34 33	ZM 7421	31 28	ZM 5021	418 00	ZM 3676	27 60
ZM 4234	870 00	897 50	27 50	251	40 85	2 13	3 00	ZM 4748	26 33	ZM 4748	33 00	ZM 7305	34 33	ZMS 606	31 67	ZM 4308	420 70	ZM 4234	24 21
ZM 4235	850 00	870 00	20 00	222	37 31	4 69	3 05	ZM 7236	26 33	ZM 7421	33 00	ZM 4310	34 50	ZM 4310	31 94	ZM 7305	421 30	ZM 4235	19 09
ZM 4236	850 00	870 00	20 00	234	43 11	5 03	3 23	ZM 4327	26 50	DKC 8033	33 33	ZM 4271	34 67	ZM 4308	32 55	ZM 4318	423 00	ZM 4236	24 98
ZM 4237	817 50	840 00	22 50	218	38 30	3 33	2 85	ZM 4249	26 67	ZMS 606	33 67	ZM 4307	34 67	ZMS 638	32 78	MM 441	429 70	ZM 4237	20 31
ZM 4245	860 00	885 00	25 00	230	30 94	3 12	3 47	ZM 5021	26 67	ZM 4253	33 83	ZM 4253	35 33	MM 501	32 78	ZM 4337	432 00	ZM 4245	13 16
Middle 20 genotypes																			
ZM 4249	850 00	870 00	20 00	220	41 11	3 65	1 91	ZM 4256	27 00	ZM 4235	34 00	ZM 4308	35 33	ZM 4327	32 83	ZM 6868	437 00	ZM 4249	22 69
ZM 4250	802 50	828 00	25 50	186	38 15	4 06	2 35	PHB 30G19	27 33	ZM 4308	34 00	ZM 4318	35 67	ZM 4324	32 89	ZM 4329	438 70	ZM 4250	41 92
ZM 4251	860 00	870 00	10 00	216	42 56	4 96	1 46	DKC 8053	27 33	ZM 4245	34 33	DKC 9089	36 00	ZM 7114	32 94	DKC 80-33	439 70	ZM 4251	38 29

Appendix 3.7.2 Continued

ZM 4252	840 00	860 00	20 00	236	31 94	3 07	1 93	ZM 6868	27 33	ZM 4255	34 33	ZM 7421	36 00	ZM 4337	33 16	ZM 4310	441 50	ZM 4252	22 00
ZM 4253	840 00	860 00	20 00	205	57 33	4 61	1 05	ZM 4234	27 83	ZM 4312	34 67	ZM 4236	36 33	ZM 4307	33 17	ZM 4748	442 30	ZM 4253	32 44
ZM 4256	817 50	840 00	22 50	209	28 86	4 69	2 53	DKC 777	28 00	ZM 4321	35 00	MM 441	36 67	ZM 4234	33 50	ZM 4321	443 70	ZM 4256	33 57
ZM 4258	817 50	840 00	22 50	211	33 76	4 14	2 74	ZM 4251	28 00	ZM 4324	35 00	ZM 4325	36 67	ZM 4323	33 89	ZM 4255	445 00	ZM 4258	37 35
ZM 4261	817 50	828 00	10 50	191	24 72	4 31	2 20	ZM 4308	28 33	ZM 7305	35 00	ZMS 606	36 67	SY 5944	33 89	SY 5944	448 70	ZM 4261	35 70
ZM 4264	788 00	817 50	29 50	210	39 68	3 99	1 92	MM 501	28 67	MM 441	35 33	MM 502	37 00	MM 441	33 89	ZM 4237	449 70	ZM 4264	38 81
ZM 4271	788 00	817 50	29 50	177	43 78	4 06	2 59	ZM 4245	28 67	PAN 7M-83	35 67	ZM 4235	37 33	MM 502	34 00	ZM 7147	453 70	ZM 4271	22 25
ZM 4307	860 00	885 00	25 00	239	28 33	4 56	2 52	ZM 4255	28 67	MM 502	35 83	MM 501	37 67	ZM 4245	34 00	ZM 4327	455 70	ZM 4307	33 35
ZM 4308	860 00	885 00	25 00	224	40 50	3 35	2 19	ZM 4264	29 00	ZM 4337	35 83	ZM 3676	37 67	ZM 4235	34 22	ZM 4256	456 00	ZM 4308	24 97
ZM 4310	870 00	897 50	27 50	214	22 96	4 61	2 38	ZM 5043	29 00	ZM 4323	36 00	ZM 4256	37 67	ZM 4329	34 22	ZM 4235	456 70	ZM 4310	21 04
ZM 4312	840 00	870 00	30 00	225	25 77	3 41	2 77	MM 502	29 17	ZMS 638	36 67	ZM 4316	37 67	7M-83	34 44	ZM 4323	463 70	ZM 4312	26 61
ZM 4316	933 50	955 00	21 50	233	17 25	3 64	2 41	ZM 4324	29 33	ZM 4310	37 00	ZM 4329	38 00	ZM 4321	34 56	ZM 4234	464 00	ZM 4316	13 79
ZM 4318	840 00	850 00	10 00	227	25 11	3 3	3 60	MM 441	29 67	ZM 4264	38 00	ZM 4258	38 67	ZM 4255	34 67	ZM 4353	466 70	ZM 4318	25 84
ZM 4321	850 00	870 00	20 00	230	27 42	3 59	2 15	ZM 4321	29 67	ZM 4353	38 00	SY 5944	39 00	ZM 4256	34 89	ZM 4245	469 30	ZM 4321	17 84
ZM 4323	870 00	908 50	38 50	223	25 02	3 57	3 07	ZM 4333	29 67	ZM 3676	38 33	ZM 4245	39 00	ZM 4236	35 22	ZM 4251	474 80	ZM 4323	15 44
ZM 4324	885 00	908 50	23 50	227	17 44	5 03	2 41	DKC 80-33	30 00	ZM 4236	38 33	ZM 4321	39 00	ZM 7147	35 33	ZM 4324	476 00	ZM 4324	19 59
ZM 4325	908 50	933 50	25 00	222	19 54	3 67	3 19	ZM 4312	30 00	ZM 4327	38 33	ZM 4353	39 00	ZM 4258	35 58	ZM 4325	477 00	ZM 4325	19 61
Bottom 20 genotypes																				
ZM 4327	933 50	955 00	21 50	233	13 49	3 59	3 53	ZM 4352	30 17	ZM 4329	38 33	ZM 4323	39 33	ZM 4325	35 67	ZM 4258	478 10	ZM 4327	9 01
ZM 4329	885 00	908 50	23 50	214	26 45	3 96	2 83	ZM 4236	31 00	ZM 4250	38 67	ZM 7147	39 33	ZM 4237	35 72	ZM 4307	480 00	ZM 4329	11 30
ZM 4333	885 00	908 50	23 50	224	23 62	4 35	2 61	ZM 4235	31 33	ZM 4325	38 67	ZM 4234	39 67	ZM 4251	35 72	ZM 4264	484 80	ZM 4333	16 08
ZM 4336	840 00	860 00	20 00	207	43 28	3 74	3 00	SY 5944	31 67	ZM 4251	39 17	ZM 4251	40 00	ZM 4264	35 72	ZMS 638	488 30	ZM 4336	18 94
ZM 4337	840 00	860 00	20 00	213	33 83	3 37	2 42	ZM 4325	31 67	ZM 4237	39 67	ZM 4333	40 00	ZM 4316	36 56	ZM 7236	489 70	ZM 4337	32 40
ZM 4342	802 50	828 00	25 50	168	21 87	4 67	1 82	ZM 4261	31 72	ZM 4352	39 67	ZM 4264	40 17	ZM 4333	36 56	ZM 4333	493 00	ZM 4342	33 89
ZM 4352	860 00	885 00	25 00	222	29 93	4 01	2 18	ZM 4307	31 83	ZM 4256	40 00	ZM 4255	41 00	ZM 4271	36 67	ZM 4250	496 00	ZM 4352	15 30
ZM 4353	885 00	908 50	23 50	230	24 11	4 03	3 72	ZMS 638	32 00	ZM 4316	40 00	ZM 4237	41 33	ZM 4353	36 67	ZM 4252	498 50	ZM 4353	10 20
ZM 4358	870 00	908 50	38 50	234	19 93	4	3 31	ZM 4250	32 00	ZM 4333	40 00	ZM 4336	41 33	ZM 4312	36 78	ZM 4316	500 30	ZM 4358	22 20
ZM 4748	828 00	840 00	12 00	183	30 11	3 62	3 21	ZM 4316	32 00	ZM 4336	40 00	ZM 5043	42 00	ZM 3676	36 81	ZM 4261	502 40	ZM 4748	28 09
ZM 5021	817 50	840 00	22 50	208	44 04	4 25	2 46	ZM 4252	32 10	ZM 7147	40 67	ZM 7114	42 33	ZM 5043	37 33	ZM 4271	502 70	ZM 5021	16 98

Appendix 3.7.2 Continued

ZM 5043	788 00	817 50	29 50	183	42 51	4 68	2 62	ZM 4337	32 33	ZM 7236	40 67	ZM 4252	42 67	ZM 4250	37 89	ZM 3676	504 90	ZM 5043	40 66
ZM 6868	885 00	908 50	23 50	232	23 02	3 35	4 29	ZM 4342	33 00	ZM 4261	41 00	ZM 4250	43 00	ZM 7236	38 11	ZM 4336	505 00	ZM 6868	10 80
ZM 7114	828 00	850 00	22 00	217	38 64	2 59	2 62	ZM 4353	33 00	ZM 4271	41 00	ZM 4261	44 50	ZM 4336	38 78	ZM 5043	506 00	ZM 7114	16 55
ZM 7147	788 00	817 50	29 50	155	21 12	2 98	1 78	ZM 4271	34 33	ZM 5043	41 00	ZM 4312	45 67	ZM 4261	39 07	ZM 4352	510 00	ZM 7147	55 02
ZM 7236	850 00	870 00	20 00	203	29 63	4 34	2 10	ZM 3676	34 43	ZM 4258	42 67	ZM 7236	47 33	ZM 4252	39 48	PAN 7M-83	513 30	ZM 7236	22 97
ZM 7305	885 00	908 50	23 50	220	39 07	3 28	2 20	7M-83	35 00	ZM 4342	43 00	ZM 4342	48 33	ZM 4352	40 06	ZM 4312	515 30	ZM 7305	11 74
ZM 7421	860 00	885 00	25 00	222	21 93	4 28	2 22	ZM 4336	35 00	ZM 4252	43 67	ZM 4352	50 33	ZM 4342	41 44	ZM 4342	536 30	ZM 7421	31 98
ZMS 606	788 00	817 50	29 50	189	21 32	2 3	7 09	MM 400	n a	MM 400	n a	MM 400	n a	MM 400	n a	MM 400	n a	ZMS 606	30 11
Mean	**836.14**	**859.63**	**23.49**	**209**	**29.18**	**3.64**	**3.45**	**Mean**	**28.68**		**35.64**		**37.18**		**33.75**				**24.16**
CV (%)	**2.17**	**2.21**	**22.14**	**7.11**	**39.11**	**25.1**	**27.93**	**CV (%)**	**14.73**		**14.01**		**16.87**		**10.34**				**51.64**
EMS	**2.06**	**2.24**	**0.19**	**0.02**	**131.33**	**0.88**	**0.95**	**EMS**	**20.06**		**28.54**		**44.63**		**11.82**				**164.43**
LSD (0.05)	**2.29**	**2.4**	**0.72**	**0.24**	**18.20**	**1.47**	**1.54**	**LSD**	**7.54**		**9.20**		**11.45**		**5.58**				**20.08**
SE	**1.43**	**1.5**	**0.36**	**0.15**	**11.38**	**0.91**	**0.95**	**SE**	**4.66**		**5.69**		**7.08**		**3.44**				**12.56**
SED	**1.17**	**1.22**	**0.03**	**0.12**	**10.47**	**0.77**	**0.80**	**SED**	**3.81**		**4.64**		**5.78**		**2.81**				**10.11**

CV = Coefficient of variation; EMS = Error mean square; LSD = Least significant difference; SE = Standard error; SED Standard Error of the mean difference; n.a. = not available, DTA = growing degree day units accumulated to reach to 50 % anthesis; DTS = growing degree day units accumulated to 50 % silking; ASI = difference in growing degree day units between DTS and DTA; PH = Plant height; ER = Ear rot reaction (%); EASP = Ear aspect on a scale of 1 to 5; GY = Grain yield (t ha^{-1}), FLD7, FLD9 and FLD10 denote FAW field damage score (%) recorded at 26, 30, and 32 days after the first FAW infestations in that order; FCD = FAW cob damage

Appendix 3.7.3. Mean values of 253 maize genotypes for agronomic traits and FAW damage parameters in Set II evaluation

	Agronomic traits						FAW parameters			
Genotype	DTA	DTS	ASI	FNP	WFC	GY	FLD1	FLD2	FLDMean	FCD
	Low-preference genotypes (based on FLDMean)									
CML304-B	888 00	918 50	30 50	2	50 23	0 10	6 25	11 50	8 87	14 42
CZL15231	625 50	658 50	33 00	1	67 45	0 13	3 74	14 96	9 33	16 03
VL05614	n a	n a	n a	3	49 47	0 34	10 85	8 72	9 75	49 60
CML486-216	645 50	658 50	13 00	9	43 51	6 07	5 29	15 69	10 45	51 64
CML442	n a	n a	n a	3	50 02	0 18	4 37	18 99	10 82	5 36
CZL16090	918 50	943 00	24 50	1	25 41	0 45	5 29	17 02	11 17	11 41

Appendix 3.7.3 Continued

TL141998	589 00	613 50	24 50	2	74 86	0 35	5 16	17 28	11 23	74 66
CML539	574 00	613 50	39 50	5	62 16	0 78	5 81	17 06	11 41	74 18
TL115627	740 50	768 00	27 50	5	79 44	0 81	11 95	11 89	11 65	46 38
TL1512847	600 50	645 50	45 00	8	52 53	1 12	7 14	17 01	12 06	45 11
CML494-76	717 00	729 00	12 00	4	89 59	0 29	12 10	12 31	12 19	47 93
CZL1368	658 50	673 00	14 50	6	39 34	0 63	6 22	18 26	12 23	27 90
CZL16015	768 00	780 00	12 00	5	90 22	1 08	9 41	15 70	12 55	77 45
CZL16093	820 00	862 50	42 50	2	49 78	0 13	9 05	16 82	12 94	74 09
CML546-B	780 00	806 00	26 00	8	54 79	1 64	10 22	16 22	13 20	62 84
TL156611	794 00	820 00	26 00	4	99 81	0 62	14 30	12 20	13 22	40 08
EBL173778	849 00	873 50	24 50	9	44 44	3 51	8 38	18 22	13 28	3 03
CZL03011	794 00	820 00	26 00	8	46 91	0 75	9 63	17 05	13 35	80 91
TL145733	794 00	820 00	26 00	6	58 14	0 64	8 67	18 05	13 37	64 86
TL1512886	564 50	589 00	24 50	2	24 65	0 80	9 58	17 30	13 43	16 29
CZL16069	849 00	862 50	13 50	3	0 03	0 27	8 16	18 89	13 48	42 65
CZL15081	862 50	888 00	25 50	3	58 44	1 05	11 56	15 51	13 54	81 91
CZL16092	849 00	873 50	24 50	4	54 61	0 65	5 48	21 63	13 55	57 99
TL133972	574 00	589 00	15 00	7	0 08	0 23	8 60	18 56	13 57	52 26
CML547	768 00	794 00	26 00	9	63 44	0 96	10 38	17 02	13 68	39 11
CML548©	820 00	849 00	29 00	2	48 79	0 25	10 77	17 30	13 81	49 19
VL058553	780 00	794 00	14 00	3	16 82	0 78	6 22	21 44	13 83	48 13
CML489	600 50	613 50	13 00	6	75 47	0 64	5 25	22 39	13 84	70 11
CML440-B	564 50	589 00	24 50	n a	0 00	0 02	9 02	19 30	13 88	7 99
CZL1347	673 00	702 50	29 50	2	100 00	0 33	9 68	18 27	13 96	53 35
EBL1611440	780 00	806 00	26 00	7	74 66	1 23	6 85	21 15	14 01	75 09
TL142036	658 50	673 00	14 50	7	67 05	1 15	6 58	21 50	14 03	64 92
CZL16136	888 00	904 50	16 50	7	72 85	0 99	8 76	19 26	14 03	81 39
TL131755	625 50	673 00	47 50	4	61 99	0 74	8 73	19 41	14 05	80 91
CZL16027	687 00	717 00	30 00	7	49 69	0 86	8 76	19 35	14 08	60 24

Appendix 3.7.3 Continued

CZL15128	564 50	589 00	24 50	5	32 94	0 64	6 64	21 65	14 15	25 79
TL116004	794 00	820 00	26 00	2	0 47	0 09	6 08	22 39	14 24	95 11
EBL1611449	873 50	888 00	14 50	5	49 82	0 40	8 50	19 50	14 26	95 96
TL142054	552 50	574 00	21 50	5	82 64	0 58	8 65	20 20	14 43	90 44
CZL16080	780 00	806 00	26 00	5	30 31	0 43	8 18	20 74	14 45	53 86
TL1512846	613 50	645 50	32 00	5	49 86	0 76	11 05	18 08	14 56	99 75
CZL15142	806 00	820 00	14 00	4	28 65	0 33	8 61	20 66	14 63	90 88
CZL16032	645 50	658 50	13 00	7	100 00	1 04	7 62	21 71	14 67	100 00
CML491	849 00	873 50	24 50	9	45 04	1 23	9 46	20 07	14 75	45 34
CML494-74	658 50	673 00	14 50	4	72 32	0 37	8 14	21 72	14 92	82 02
CZL16137	613 50	625 50	12 00	2	74 86	0 23	7 17	22 77	14 95	45 82
Moderate-preference genotypes (based on FLDMean)										
SYN312-236	849 00	873 50	24 50	9	34 78	1 22	11 71	18 52	15 11	24 76
CZL15122	873 50	888 00	14 50	3	41 74	0 39	7 96	22 40	15 15	88 37
TL101644	552 50	574 00	21 50	11	97 30	5 98	9 28	21 18	15 20	97 50
CML548-B	574 00	600 50	26 50	9	84 99	3 20	9 79	20 91	15 27	73 50
CZL16099	806 00	820 00	14 00	5	71 13	0 69	12 88	17 70	15 30	81 27
TL155942	849 00	873 50	24 50	2	0 11	0 30	12 56	18 23	15 38	96 44
CZL15224	806 00	820 00	14 00	4	83 45	1 03	8 03	22 92	15 45	56 40
CZL16025	849 00	873 50	24 50	8	60 42	1 11	9 29	21 68	15 49	37 59
TL115657	740 50	768 00	27 50	6	75 29	0 69	10 79	19 64	15 52	69 84
CZL16060	862 50	873 50	11 00	6	51 84	0 99	9 20	21 94	15 55	32 90
EBL173774	780 00	806 00	26 00	7	47 83	1 26	9 36	21 80	15 57	100 00
CZL1310c	645 50	673 00	27 50	5	37 21	0 46	11 57	19 74	15 63	26 55
TL1512864	702 50	754 00	51 50	4	62 93	0 40	12 89	18 52	15 68	100 00
CML536	780 00	806 00	26 00	7	72 37	0 82	7 53	23 83	15 69	85 59
TL148266	658 50	687 00	28 50	4	63 69	0 41	6 49	24 89	15 71	86 40
CZL15194	806 00	849 00	43 00	5	87 72	0 55	10 05	21 41	15 71	72 36
CZL15202	849 00	873 50	24 50	3	50 06	0 38	5 32	26 23	15 78	79 68

Appendix 3.7.3 Continued

CZL16184	658 50	687 00	28 50	4	50 49	0 29	7 51	24 11	15 80	92 23
CZL16100	740 50	768 00	27 50	6	99 51	1 45	11 06	20 57	15 81	98 39
CZL15181	613 50	645 50	32 00	6	66 40	0 64	9 14	22 55	15 86	55 47
CZL15076	613 50	645 50	32 00	2	25 30	0 35	9 19	22 60	15 90	72 04
TL115741	873 50	888 00	14 50	5	0 00	0 34	8 39	23 40	15 90	68 85
CML537	768 00	794 00	26 00	6	48 88	0 47	13 79	18 15	15 98	36 34
TL139200	600 50	625 50	25 00	4	37 56	0 19	7 85	24 20	16 03	47 44
CZL15209	768 00	768 00	0 00	3	49 46	0 20	9 50	22 55	16 03	86 89
CZL16177	794 00	806 00	12 00	3	65 77	0 29	9 08	23 09	16 06	86 84
CZL15222	862 50	873 50	11 00	4	80 10	0 73	10 09	22 12	16 11	36 19
TL13159	645 50	658 50	13 00	8	79 57	1 32	9 85	22 36	16 12	80 42
TL1512861	873 50	904 50	31 00	6	62 77	1 32	7 49	24 75	16 14	77 63
CZL16063	740 50	768 00	27 50	5	66 14	0 40	10 39	22 05	16 23	53 73
CZL16061	849 00	862 50	13 50	4	49 33	0 39	6 65	25 80	16 23	34 72
CZL052	780 00	806 00	26 00	9	19 54	0 72	8 36	24 18	16 25	53 04
CML494-78	729 00	754 00	25 00	1	n a	n a	5 04	27 59	16 27	n a
TL173	613 50	625 50	12 00	7	88 64	1 83	7 41	25 12	16 29	49 08
CZL15083	780 00	794 00	14 00	4	75 43	1 25	7 84	24 86	16 34	57 00
TL1512841	645 50	673 00	27 50	6	47 44	0 96	12 11	20 55	16 35	41 03
TL1611604	780 00	806 00	26 00	4	68 61	1 72	7 51	25 16	16 37	52 75
CKL05024	862 50	873 50	11 00	6	71 07	0 92	7 77	24 93	16 38	20 81
TL1611608	849 00	873 50	24 50	4	56 20	0 72	9 27	23 51	16 40	44 29
CZL15033	673 00	702 50	29 50	7	35 48	0 90	10 38	22 49	16 45	100 00
CML488	625 50	658 50	33 00	4	33 95	0 45	8 37	24 18	16 46	100 00
CZL16095	574 00	600 50	26 50	4	40 30	0 52	8 34	24 60	16 47	66 14
CZL15173	687 00	702 50	15 50	6	66 56	0 37	9 47	23 61	16 54	64 60
CML538	820 00	862 50	42 50	1	24 40	0 15	9 87	23 23	16 55	73 09
EBL1611462	862 50	873 50	11 00	5	66 69	0 45	11 63	21 46	16 55	61 49
EBL173777	820 00	849 00	29 00	7	57 66	0 63	14 23	18 92	16 56	64 53

Appendix 3.7.3 Continued

CZL15178	n a	n a	n a	5	83 51	0 92	8 11	25 02	16 56	100 00
CML537-108	780 00	806 00	26 00	2	0 00	0 17	7 93	25 20	16 57	46 81
CML495	673 00	702 50	29 50	8	72 56	1 17	11 31	21 83	16 58	48 34
CZL16029	n a	n a	n a	4	41 89	0 21	7 00	26 21	16 58	72 02
CZL16084	687 00	717 00	30 00	8	63 67	0 83	10 86	22 31	16 60	56 27
CML445	780 00	806 00	26 00	5	99 64	0 83	11 47	21 79	16 63	81 55
TL148348	645 50	658 50	13 00	3	33 24	0 07	7 20	26 25	16 70	100 00
CML441-B	658 50	673 00	14 50	5	50 37	1 21	10 94	22 45	16 72	31 25
TL1611611	768 00	794 00	26 00	4	25 80	0 04	7 34	26 35	16 84	80 09
TL115743	768 00	794 00	26 00	6	52 08	1 18	9 53	24 12	16 86	71 03
CKDHL0323	794 00	806 00	12 00	6	48 16	0 56	10 39	23 37	16 86	24 35
CZL16181	625 50	658 50	33 00	8	0 00	0 09	7 49	26 28	16 88	100 00
CZL15123	768 00	794 00	26 00	5	54 91	1 16	8 91	24 89	16 93	90 49
TL115679	873 50	888 00	14 50	3	82 63	0 29	10 97	22 96	16 97	66 55
CZL1117	600 50	625 50	25 00	7	58 02	0 30	9 91	24 06	16 99	12 87
TL142183	574 00	589 00	15 00	2	49 63	0 19	6 83	27 24	17 03	98 12
CZL1423	806 00	820 00	14 00	4	66 93	0 30	8 31	25 87	17 08	27 60
TL116163	768 00	780 00	12 00	6	39 31	0 54	10 25	23 90	17 08	45 41
CZL16075	820 00	873 50	53 50	6	45 48	0 61	13 16	21 07	17 09	26 49
CZL1011c	613 50	645 50	32 00	4	45 22	0 44	8 00	26 20	17 10	70 26
CZL15220	780 00	806 00	26 00	7	73 52	0 62	8 00	26 32	17 17	34 51
CZL16176	820 00	862 50	42 50	4	36 08	0 44	7 92	26 41	17 18	52 28
TL1611615	754 00	780 00	26 00	2	16 39	0 19	8 86	25 57	17 18	100 00
TL123268	780 00	806 00	26 00	2	25 38	0 21	9 55	24 88	17 20	51 20
TL12176	645 50	673 00	27 50	10	33 92	0 71	12 51	21 89	17 22	56 92
CML444-176	687 00	754 00	67 00	8	43 66	3 63	9 63	24 86	17 25	25 93
TL142012	740 50	754 00	13 50	3	41 43	0 16	9 47	25 05	17 26	30 32
CML451-215	849 00	873 50	24 50	5	49 35	1 58	6 69	27 88	17 28	33 71
CML539-113	862 50	873 50	11 00	7	47 17	0 72	9 22	25 42	17 31	40 46

Appendix 3.7.3 Continued

TL156610	849 00	873 50	24 50	4	99 49	0 49	11 61	23 04	17 32	27 57
CZL15119	849 00	873 50	24 50	2	74 61	0 36	10 04	24 56	17 32	100 00
TL139251	820 00	862 50	42 50	7	30 42	0 64	10 15	24 48	17 32	100 00
CZL15228	645 50	658 50	13 00	6	41 58	0 55	8 47	26 31	17 36	78 66
TL115786	849 00	873 50	24 50	4	62 04	0 99	7 28	27 47	17 41	22 01
CZL1463	564 50	589 00	24 50	1	73 71	0 20	11 14	23 75	17 46	99 52
check 3	820 00	862 50	42 50	12	66 51	4 82	10 83	24 14	17 48	50 96
CML548 (C)	820 00	862 50	42 50	3	33 80	0 38	7 52	26 89	17 48	28 44
EBL173776	564 50	586 00	21 50	10	76 03	5 09	9 69	25 31	17 52	53 56
CZL15234	687 00	717 00	30 00	4	82 64	0 37	12 64	22 40	17 53	50 91
CML537-106	849 00	862 50	13 50	3	74 54	0 35	7 85	27 27	17 55	100 00
TL1611554	873 50	888 00	14 50	3	33 50	0 34	9 89	25 19	17 56	63 83
CZL15205	625 50	645 50	20 00	4	0 20	0 54	9 22	26 01	17 59	63 94
CZL16049	754 00	768 00	14 00	2	75 61	0 29	8 70	26 51	17 64	25 55
CML572	780 00	806 00	26 00	4	24 53	0 39	6 63	28 86	17 76	100 00
CZL16134	625 50	645 50	20 00	3	58 81	0 17	9 94	25 64	17 78	66 59
VL05617	574 00	613 50	39 50	3	100 00	0 45	9 99	25 60	17 80	85 53
CML571	780 00	794 00	14 00	8	75 18	1 05	9 38	26 30	17 85	84 47
TL1611609	794 00	820 00	26 00	6	38 10	0 55	9 34	26 44	17 85	90 48
CML312-2	849 00	862 50	13 50	8	74 53	6 53	11 23	24 57	17 87	9 62
CML496-222	780 00	806 00	26 00	5	53 15	0 97	6 36	29 40	17 88	65 31
CML540-B	589 00	625 50	36 50	6	63 71	1 49	9 41	26 54	17 98	52 93
CZL1466	768 00	806 00	38 00	8	24 49	0 50	11 40	24 64	18 03	54 37
CZL1350	625 50	658 50	33 00	3	49 55	0 23	9 79	26 27	18 06	98 21
CZL15127	754 00	780 00	26 00	4	50 61	0 84	9 32	26 79	18 07	36 51
TL142179	645 50	658 50	13 00	4	74 73	0 72	11 50	24 70	18 10	99 21
CZL15237	613 50	645 50	32 00	5	33 19	0 18	7 51	28 74	18 14	92 87
TL123332	862 50	888 00	25 50	4	0 18	0 10	12 82	23 58	18 20	35 38
TL1313	589 00	600 50	11 50	3	50 19	0 17	9 16	27 34	18 22	72 44

Appendix 3.7.3 Continued

CZL16147	589 00	645 50	56 50	3	74 82	0 43	13 27	23 13	18 23	51 78
CZL111	687 00	702 50	15 50	3	62 70	0 41	12 41	24 10	18 26	74 00
EBLI611449	729 00	740 50	11 50	7	28 10	0 49	11 04	26 13	18 34	91 86
EBL1611436	849 00	873 50	24 50	4	100 00	0 25	6 60	30 18	18 38	68 08
Laposta-141	794 00	806 00	12 00	7	49 64	0 36	11 04	25 73	18 41	82 20
TL139178	780 00	806 00	26 00	8	83 01	0 39	10 14	26 82	18 47	64 34
check 2	849 00	873 50	24 50	9	79 85	2 37	10 97	25 96	18 47	35 01
CZL16067	754 00	780 00	26 00	4	49 64	0 78	13 49	23 49	18 50	83 50
EBL167726	794 00	806 00	12 00	4	43 20	0 44	7 32	29 88	18 58	69 38
CL147013	918 50	932 50	14 00	2	25 53	0 13	9 37	27 84	18 62	24 31
TL142139	794 00	806 00	12 00	6	74 34	0 65	12 24	25 06	18 63	84 29
TL142151	849 00	873 50	24 50	4	62 61	0 35	8 57	29 02	18 78	97 64
CZL16017	702 50	729 00	26 50	1	89 59	3 60	12 70	24 94	18 80	9 17
CZL1358	820 00	862 50	42 50	7	34 88	0 45	9 77	27 90	18 82	86 36
CML491-71	862 50	888 00	25 50	4	70 62	0 54	4 60	33 05	18 83	60 34
TL139155	625 50	687 00	61 50	3	87 90	0 47	11 56	26 19	18 85	67 02
TL132023	625 50	658 50	33 00	6	24 84	0 27	9 62	28 11	18 89	57 07
CML539-116	820 00	862 50	42 50	9	32 77	0 63	14 22	23 64	18 93	81 18
CZL16035	918 50	943 00	24 50	3	74 98	0 08	9 57	28 39	18 95	81 32
CZL0712	780 00	794 00	14 00	4	50 21	0 33	9 05	28 87	18 98	76 74
CML545-B	552 50	574 00	21 50	6	85 58	0 98	9 17	28 76	18 99	81 60
CZL16170	862 50	888 00	25 50	6	68 38	0 95	14 27	23 80	19 04	77 15
CML395	589 00	600 50	11 50	4	75 52	0 42	15 53	22 53	19 05	51 68
CZL16132	754 00	780 00	26 00	4	24 76	0 81	8 82	29 24	19 05	63 98
CZL15168	768 00	780 00	12 00	5	0 00	0 36	11 29	26 82	19 09	98 96
CML443	717 00	780 00	63 00	6	44 54	0 59	11 89	26 36	19 12	54 62
INTA181	645 50	673 00	27 50	4	32 82	0 27	8 39	29 84	19 13	41 51
CZL16121	768 00	780 00	12 00	3	67 66	0 33	8 44	29 96	19 17	96 68
CZL15077				5	32 66	0 25	10 99	27 35	19 18	96 03

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CZL16016	613 50	645 50	32 00	5	74 90	0 54	13 88	24 62	19 26	78 87
VL081463	564 50	589 00	24 50	3	44 64	0 21	11 23	27 26	19 27	64 84
TL1512891	645 50	658 50	13 00	6	79 24	0 84	9 72	28 90	19 29	81 00
EBL173764	806 00	820 00	14 00	4	57 88	0 24	12 30	26 34	19 32	90 20
TL1316	717 00	729 00	12 00	7	93 62	3 50	12 99	25 66	19 32	63 59
TL145671	849 00	873 50	24 50	3	50 92	0 08	8 93	29 78	19 34	99 28
CZL15130				1	0 29	0 10	13 87	24 73	19 35	94 84
CZL16091	862 50	888 00	25 50	5	50 43	0 59	11 55	27 17	19 38	40 11
CZL16107	754 00	780 00	26 00	5	n a	n a	7 87	30 94	19 41	n a
EBL1611442	645 50	658 50	13 00	7	83 89	1 12	11 69	27 18	19 42	74 14
CZL16138	820 00	862 50	42 50	5	89 59	0 70	11 86	27 06	19 43	57 80
VL055011	625 50	645 50	20 00	5	20 17	1 04	8 00	30 38	19 46	17 16
EBL1611469	768 00	794 00	26 00	5	82 67	0 37	4 23	34 76	19 48	78 93
CML547-B	820 00	862 50	42 50	7	53 67	2 19	8 82	30 17	19 50	100 00
CZL16101	613 50	645 50	32 00	6	91 83	0 91	10 07	28 98	19 52	99 42
TL1512869	625 50	645 50	20 00	2	71 58	0 44	9 90	29 19	19 53	28 95
TL1512845	794 00	820 00	26 00	5	88 85	0 61	13 73	25 34	19 54	77 12
CZL16051	862 50	888 00	25 50	2	25 80	0 22	8 55	30 68	19 60	74 40
CZL15225	645 50	687 00	41 50	4	46 16	0 39	7 47	32 06	19 74	73 95
CZL04016	658 50	673 00	14 50	2	58 03	0 09	9 28	30 42	19 85	82 84
TL116067	794 00	820 00	26 00	4	30 13	0 19	8 99	30 81	19 87	69 12
CZL16013	806 00	849 00	43 00	6	58 62	0 83	10 90	29 04	19 96	74 95
CZL16143	552 50	564 50	12 00	2	75 52	0 13	12 77	27 24	20 01	49 25
CZL16098	645 50	673 00	27 50	5	75 75	0 29	11 57	28 47	20 02	100 00
CML444-55	687 00	702 50	15 50	5	99 71	0 09	13 58	26 53	20 03	52 75
CKDHL0089-B	820 00	862 50	42 50	1	49 44	0 19	5 60	34 15	20 15	6 38
TL1611607	673 00	702 50	29 50	6	79 43	0 92	11 35	28 99	20 17	51 63
CZL068	673 00	687 00	14 00	7	90 35	1 14	7 60	32 73	20 17	50 66
EBL173810	794 00	820 00	26 00	7	82 78	0 96	12 01	28 45	20 25	37 85

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CML539-114	820 00	888 00	68 00	6	52 25	0 60	11 44	29 21	20 34	65 50
check 1	768 00	794 00	26 00	9	82 86	3 72	13 54	27 24	20 39	63 41
CML494	625 50	645 50	20 00	5	61 15	1 08	12 29	28 48	20 39	65 26
CZL15006	702 50	729 00	26 50	6	53 23	0 09	12 48	28 41	20 44	55 80
CML546	794 00	820 00	26 00	8	60 05	1 04	15 22	25 70	20 46	51 17
CZL16141	658 50	687 00	28 50	7	85 74	0 72	14 44	26 57	20 48	70 51
CZL1349	862 50	888 00	25 50	4	71 08	0 26	13 01	28 07	20 54	76 32
CZL16173	862 50	873 50	11 00	6	75 45	0 90	11 16	29 89	20 55	89 34
CZL1369	574 00	589 00	15 00	6	79 80	1 36	12 97	28 10	20 57	81 98
TL139180	820 00	862 50	42 50	4	100 00	0 52	10 63	30 62	20 62	84 25
TL1611610	794 00	820 00	26 00	5	51 18	0 48	8 64	32 60	20 62	60 67
CZL16045	740 50	768 00	27 50	7	41 83	0 26	11 28	29 98	20 65	28 56
TL142140	849 00	873 50	24 50	5	25 40	0 17	16 15	25 35	20 76	97 98
CKDHL0228	849 00	873 50	24 50	7	60 52	1 03	8 32	33 29	20 80	42 84
TL1611603	794 00	820 00	26 00	8	19 70	0 26	12 87	28 75	20 83	78 69
CZL15192	645 50	673 00	27 50	4	62 71	0 39	11 51	30 14	20 84	87 23
CML312	873 50	904 50	31 00	6	57 94	1 83	10 47	31 42	20 93	42 02
High -preference genotypes (based on FLD Mean)										
CML543	702 50	729 00	26 50	4	41 62	0 08	12 02	30 13	21 06	20 21
CZL16043	820 00	862 50	42 50	5	7 59	0 45	12 20	30 07	21 14	40 03
CML537-102	645 50	673 00	27 50	6	65 71	0 97	8 13	34 31	21 20	84 93
CZL1354	873 50	904 50	31 00	4	0 12	0 05	7 37	35 10	21 22	0 44
CZL16139	849 00	862 50	13 50	5	0 94	0 25	14 72	28 51	21 34	57 20
CZL1432	806 00	849 00	43 00	4	70 17	0 39	12 11	30 70	21 40	42 09
VL056281	780 00	806 00	26 00	4	15 98	0 20	14 11	29 10	21 60	91 97
CZL1211	625 50	645 50	20 00	3	67 26	0 13	7 15	36 21	21 72	100 00
ZEWA-239	552 50	564 50	12 00	3	25 31	0 15	9 66	34 07	21 85	97 02
CML197-B	873 50	888 00	14 50	7	84 78	1 03	13 61	30 36	21 97	7 20
TL142017	552 50	574 00	21 50	5	75 39	0 29	13 21	31 05	22 15	73 86

Appendix 3.7.3 Continued

CZL16106	740 50	768 00	27 50	1	98 79	0 32	10 54	33 88	22 23	99 19
CZL13104	849 00	862 50	13 50	6	37 25	0 16	11 40	32 98	22 23	64 44
CZL0710	820 00	862 50	42 50	3	25 56	0 19	8 00	36 82	22 37	78 40
CZL15103	673 00	702 50	29 50	7	57 33	0 42	16 03	29 60	22 79	76 99
TL1512849	658 50	658 50	0 00	2	83 71	0 36	12 01	33 68	22 85	42 59
CZL1360	794 00	820 00	26 00	4	36 66	0 36	8 67	37 68	23 15	30 42
TL1315	552 50	574 00	21 50	4	37 96	0 52	12 97	33 90	23 45	19 56
CML144-246	n a	n a	n a	1	1 69	0 13	10 80	36 17	23 50	n a
TL1611613	849 00	862 50	13 50	6	33 71	0 91	15 81	31 29	23 53	73 76
TL1512887	564 50	589 00	24 50	3	67 15	0 19	15 28	32 03	23 66	74 00
CZL1346	625 50	658 50	33 00	5	17 05	0 32	13 26	34 58	23 94	100 00
EBL169550	687 00	702 50	15 50	6	54 52	1 56	19 42	28 75	24 11	77 56
CZL13100	754 00	820 00	66 00	5	37 94	0 57	10 57	37 95	24 23	89 41
TL155952	849 00	873 50	24 50	1	99 65	0 18	7 66	41 02	24 35	99 40
CZL15167	862 50	888 00	25 50	3	9 62	0 19	12 22	36 65	24 47	66 38
CML444	873 50	888 00	14 50	4	10 00	0 69	10 20	40 62	25 40	47 58
VL054530	862 50	873 50	11 00	2	98 81	0 11	8 85	42 46	25 62	100 00
CZL16026	n a	n a	n a	7	50 56	0 29	10 12	44 63	27 42	50 72
CZL055011	625 50	645 50	20 00	5	32 87	0 67	9 04	48 13	28 34	56 15
TL122165	n a	n a	n a	4	0 00	0 25	n a	n a	n a	7 20
Mean	739 05	763 92	24 88	5	54 40	0 75	9 87	25 45	17 65	62 81
CV (%)	2 55	2 76	34 81	29 17	50 53	40 25	31 04	25 55	21 09	44 76
LSD (0 05)	3 48	3 86	1 70	2 95	57 45	0 64	6 14	12 98	7 43	57 75
S E	1 78	1 97	0 61	1 43	28 03	0 38	3 07	6 50	3 72	28 17
SED	1 81	1 99	0 60	1 49	29 11	0 31	3 22	6 81	3 90	29 96

CV = Coefficient of variation; EMS = Error mean square; LSD = Least significant difference; SE = Standard error; SED = Standard Error of the mean difference; n a = not available; DTA = growing degree day units accumulated to reach to 50 % anthesis; DTS = growing degree day units accumulated to 50 % silking; ASI = difference in growing degree day units between DTS and DTA FNP = Final number of plants; WFC = Well-filled cobs; GY = Grain yield (t ha^{-1}) FLD1- Early-whorl stage FAW damage; FLD2 = Mid-whorl stage FAW damage; FLDMean = average of FLD1 and FLD2; FCD = FAW cob damage

Appendix 3.7.4. Genotype codes used in the bi-plot analysis for Set I evaluation

Genotype	Code	Genotype	Code	Genotype	Code
DKC 80-53	E1	MM 502	E21	ZM 4251	E41
DKC 777	E2	ZM 4323	E22	ZM 4327	E42
Pool 16	E3	ZM 4318	E23	ZM 4352	E43
ZM 4358	E4	ZM 4308	E24	ZMS 638	E44
ZM 7114	E5	ZM 4329	E25	ZM 4271	E45
ZM 5021	E6	ZM 4234	E26	ZM 4353	E46
MM 501	E7	ZM 7305	E27	ZM 7147	E47
DKC 9089	E8	ZM 4253	E28	ZM 3676	E48
ZM 6868	E9	ZM 4307	E29	ZM 4256	E49
PHB 30G19	E10	PAN 7M-83	E30	ZM 4261	E50
SY5944	E11	ZM 4337	E31	ZM 7236	E51
ZM 4748	E12	ZM 4310	E32	ZM 4316	E52
ZM 7421	E13	ZM 4236	E33	ZM 4252	E53
ZM 4249	E14	ZM 4237	E34	ZM 4342	E54
ZM 4235	E15	ZM 4324	E35	ZM 4258	E55
DKC 80-33	E16	ZM 4264	E36	ZM 4336	E56
MM 4255	E17	ZM 4245	E37	ZM 4333	E57
ZM 4321	E18	ZM 4312	E38	ZM 5043	E58
MM 441	E19	ZM 4250	E39	-	-
ZMS 606	E20	ZM 4325	E40	-	-

- not available

Appendix 3.7.5. Genotype codes used in the bi-plot analysis for Set II evaluation

Genotype	Code	Genotype	Code	Genotype	Code	Genotype	Code	Genotype	Code	Genotype	Code	Genotype	Code	Genotype	Code
CML304-B	E1	CML489	E28	TL115657	E55	TL1512841	E82	CZL1423	E109	CML572	E136	CZL16017	E163	CZL16138	E190
CZL15231	E2	CML440-B	E29	CZL16060	E56	TL1611604	E83	TL156610	E110	CZL16134	E137	CZL1358	E164	VL055011	E191
VL05614	E3	CZL1347	E30	EBL173774	E57	CKL05024	E84	CZL15119	E111	VL05617	E138	CML491-71	E165	EBL1611469	E192

Appendix 3.7.5 Continued

CML486-216	E4	EBL1611440	E31	CZL1310c	E58	TL1611608	E85	TL139251	E112	CML571	E139	TL139155	E166	CML547-B	E193
CML442	E5	TL142036	E32	TL1512864	E59	CZL15033	E86	CZL15228	E113	TL1611609	E140	TL132023	E167	CZL16101	E194
CZL16090	E6	CZL16136	E33	CML536	E60	CML488	E87	TL115786	E114	MIS205	E141	CML539-114	E168	TL1512869	E195
TL141998	E7	TL131755	E34	TL148266	E61	CZL16095	E88	CZL1463	E115	MIS222	E142	CZL16035	E169	TL1512845	E196
CML539	E8	CZL16027	E35	CZL15194	E62	CZL15173	E89	check 3	E116	CML540-B	E143	CZL0712	E170	CZL16051	E197
TL115627	E9	CZL15128	E36	CZL15202	E63	CML538	E90	CML548 (C)	E117	CZL1466	E144	CML545-B	E171	CZL15225	E198
TL1512847	E10	TL116004	E37	CZL16184	E64	EBL1611462	E91	CML444-176	E118	CZL1350	E145	CZL16170	E172	CZL04016	E199
CML494-76	E11	EBL1611449	E38	CZL16100	E65	EBL173777	E92	TL142012	E119	CZL15127	E146	CML395	E173	TL116067	E200
CZL1368	E12	TL142054	E39	CZL15181	E66	CZL15178	E93	CML451-215	E120	TL142179	E147	CZL16132	E174	CZL16013	E201
CZL16015	E13	CZL16080	E40	CZL15076	E67	CML537-108	E94	CML539-113	E121	CZL15237	E148	CZL15168	E175	CZL16143	E202
CZL16093	E14	TL1512846	E41	TL115741	E68	CML495	E95	TL156610	E122	TL123332	E149	CML443	E176	CZL16098	E203
CML546-B	E15	CZL15142	E42	CML537	E69	CZL16029	E96	CZL15119	E123	TL1313	E150	INTA-181	E177	CZL068	E204
TL156611	E16	CZL16032	E43	TL139200	E70	CZL16084	E97	TL139251	E124	CZL16147	E151	CZL16121	E178	CKDHL0089-B	E205
EBL173778	E17	CML491	E44	CZL15209	E71	CML445	E98	CZL15228	E125	CZL111	E152	CZL15077	E179	TL1611607	E206
CZL03011	E18	CML494-74	E45	CZL16177	E72	TL148348	E99	TL115786	E126	EBLI611449	E153	CZL16016	E180	CZL068	E207
TL145733	E19	CZL16137	E46	CZL15222	E73	CML441-B	E100	CZL1463	E127	EBL1611436	E154	VL081463	E181	EBL173810	E208
TL1512886	E20	SYN312-236	E47	TL13159	E74	TL1611611	E101	check 3	E128	Laposta-141	E155	TL1512891	E182	CML539-114	E209
CZL16069	E21	CZL15122	E48	TL1512861	E75	TL115743	E102	CML548 (C)	E129	TL139178	E156	EBL173764	E183	check 1	E210
CZL15081	E22	TL101644	E49	CZL16063	E76	CKDHL0323	E103	EBL173776	E130	check 2	E157	TL1316	E184	CML494	E211
CZL16092	E23	CML548-B	E50	CZL16061	E77	CZL16181	E104	CZL15234	E131	CZL16067	E158	TL145671	E185	CZL15006	E212
TL133972	E24	CZL16099	E51	CZL052	E78	TL12176	E105	MIS106	E132	EBL167726	E159	CZL15130	E186	CML546	E213
CML547	E25	TL155942	E52	CML494-78	E79	CML444-176	E106	TL1611554	E133	CL147013	E160	CZL16091	E187	CZL16141	E214
CML548©	E26	CZL15224	E53	TL173	E80	TL142012	E107	CZL15205	E134	TL142139	E161	CZL16107	E188	CZL1349	E215
VL058553	E27	CZL16025	E54	CZL15083	E81	CML451-215	E108	CZL16049	E135	TL142151	E162	EBL1611442	E189	CZL16173	E216
CZL1369	E217	CKDHL0228	E222	CZL16043	E227	VL056281	E232	CZL16106	E237	CZL1360	E242	CZL13100	E249	CZL16026	E254
TL139180	E218	TL1611603	E223	CML537-102	E228	CZL1211	E233	CZL13104	E238	TL1315	E243	TL155952	E250	CZL055011	E255
TL1611610	E219	CZL15192	E224	CZL1354	E229	ZEWA-239	E234	CZL0710	E239	TL1512887	E246	CZL15167	E251	TL122165	E256
CZL16045	E220	CML312	E225	CZL16139	E230	CML197-B	E235	CZL15103	E240	CZL1346	E247	CML444	E252	n a	n a
TL142140	E221	CML543	E226	CZL1432	E231	TL142017	E236	TL1512849	E241	EBL169550	E248	VL054530	E253	n a	n a

Appendix 3.7.6. List and pedigrees of the selected 30 genotypes.

Genotype name	Type	Pedigree/Source
Set I genotypes		
ZM 4236	Landrace	Southern province
ZM 7114	Landrace	North-western province
MM 501	Single cross	ZARI
MM 502	Single cross	ZARI
Pool 16	Open pollinated variety (OPV)	ZARI
Set II genotypes		
CML539	Inbred line	CML539-B
TL1512847	Inbred line	(Syn01E2-64-2-B-2-B/CIMCALI8843/S9243-BB-#-B-5-1-BB-4-3-4-B)-B-14-1-2-2-B-B-B
CZL16015	Inbred line	(La Posta Seq C7-F96-1-2-1-1-B-B-B/CML444/CML444) DH-16-B-B-B-B-B-B
CZL1347	Inbred line	(CML509/[INTA-2-1-3/INTA-60-1-2]-X-11-6-3-BBB)F2-216-3-2-B-B-B-B
CML491	Inbred line	CML491-B
CZL16137	Inbred line	(ZM621A-10-1-1-1-2-B*10/[CML390/CML206]-BB-2-4-B*7//[ZM621A-10-1-1-1-2-B*7/MAS[MSR/312]-117-2-2-1-B*5]-B-8-4-1)-B-1-2-6-B-B-B-B
CML548-B	Inbred line	CML548-B
CZL1310c	Inbred line	[[MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-Sn/GQL5]-B-23-4-1-B*5-B-B-4-B
CZL15209	Inbred line	(CML540/CML545)-B-B-B-6-2-B
TL13159	Inbred line	(ZEWAc2F2-183-2-BBB-B/[INTA-2-1-3/INTA-60-1-2]-X-11-6-3-BBB-B)DH-43-B-9-B
TL173	Inbred line	[(CML395/CML444)-B-4-1-3-1-B/CML395//DTPWC8F31-1-1-2-2]-5-1-2-2-B*4-B-5-B
CZL16095	Inbred line	([CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB//INTA-F2-192-2-1-1-1-BBBB]-1-5-1-1-1-BBB-B-B-B*/OFP106)-1-1-5-2-2-B-B-B
TL116163	Inbred line	00SADVEB-#-74-1-1-1-1-1-B-B
CZL15220	Inbred line	([INTA-2-1-3/INTA-60-1-2]-X-11-6-3/La Posta Seq C7-F64-2-6-2-2-B)-B-18-2-2-2-B-B-11-B
TL12176	Inbred line	(CML537*/OFP106)-5-2-1-1-2-B-B
CZL15234	Inbred line	(CML505/La Posta Seq C7-F71-1-2-1-2-B)-B-2-1-2-1-B-B-4-B
CZL1466	Inbred line	(CML539*/OFP14)-2-1-3-1-1-B-B-4-B
TL142139	Inbred line	(CML546/CML511)-B-B-B-2-3-B
TL142151	Inbred line	(CML546/CML511)-B-B-B-2-2-B

Appendix 3.7.6 Continued

CML545-B	Inbred line	CML545-B
CZL16016	Inbred line	(CML505/[INTA-2-1-3/INTA-60-1-2]-X-11-6-3-B)DH-3020-B-13-B
TL1316	Inbred line	([CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB//INTA-F2-192-2-1-1-1-B*4]-1-5-1-2-1-B*6/CML505)DH-2-B-B
CZL1369	Inbred line	00SADVEB-#-17-2-1-1-1-B-B
TL139180	Inbred line	(CML202/CML204)DH-3018-B
CZL 15033	Inbred line	[[KILIMA(ST94)-S5:101/CML442]-BB-1-1/CML390]-3-3-1-2-1-B-B

CHAPTER 4. SCREENING FOR FALL ARMYWORM RESISTANCE IN MAIZE: METHODOLOGIES FOR CONTROLLED EVALUATION AND GENOTYPE SELECTION

Abstract

The fall armyworm **(**FAW) (*Spodoptera frugiperda* J.E. Smith) is a newly invasive pest that threatens maize production and productivity in Africa. However, with FAW having only arrived recently in the continent, there have been no programs dedicated to breeding resistance to FAW in locally adapted maize varieties. Further, there is limited understanding of the pest's biology and development, and no information on the optimised artificial rearing of FAW, protocols to artificially infest maize crops with FAW as basis for controlled genotype screening and variety development. Multi-disciplinary research and the development of standardised FAW screening methodologies are key to the development of effective and integrated management strategies based on FAW resistant cultivars. The objectives of this study were: 1) To investigate the biology and development of FAW; 2) To develop a standardised methodology for laboratory rearing of FAW; and 3) To evaluate the efficacy of artificial infestation of FAW using selected maize genotypes, for the identification of FAW resistant maize genotypes under controlled conditions for resistance breeding. Field-collected FAW egg masses and larvae were used to initiate fresh colonies of larvae and eggs for laboratory rearing of the FAW. About 30 field-collected egg masses and 60 larvae were sampled, transferred and reared in Petri dishes using a natural maize leaf- and stalk-based diet, and an artificial soy and wheat flour-based diet in the laboratory at a temperature of 27 ± 1°C and relative humidity of 60 ± 5%. Neonate larvae that resulted from the egg masses were separated into two sets, A and B, and maintained on either the natural or artificial diet. Set A of the neonates, together with the field-collected larvae, were visually monitored for the duration of their life cycle and subsequently mated to produce a new generation of FAW. Neonates in Set B were inoculated on 63 single-cross hybrids and open pollinated maize varieties at the third and fourth maize vegetative growth stages using an artist's paint brush with six neonates per plant. Over 2000 FAW larvae were generated for inoculation of test genotypes. Inoculated maize genotypes revealed differential FAW reaction types, confirming the efficacy of the test conditions. Several genotypes, including TL13159, TL02562, TL142151, VL050120, and CML548-B, exhibited a strong resistance reaction to the FAW, while CML545-B, CZL1310c, CZL16095, EBL169550, ZM4236 and Pool 16 displayed moderate resistance. The current study enabled an understanding of the salient features of FAW growth and development under local

conditions, which may help to implement integrated FAW management strategies. The study established a standardised laboratory and screenhouse-based protocol for the mass rearing and artificial infestation of FAW onto maize plants to reliably screen maize genotypes for resistance breeding programs in Zambia or other sub-Saharan Africa countries.

Key words: fall armyworm, inoculation, life-cycle stages, rearing methods, maize screening, host plant resistance

4.1 Introduction

The fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith) is a highly destructive pest of maize (Abrahams et al., 2017). The presence of the pest in over 40 countries in Africa has combined with existing production constraints threatening maize yields, and is now risking the livelihoods of over 500 million people who depend on maize production and consumption (Macauley and Ramadjita, 2015; CABI 2019). In sub-Saharan Africa (SSA), FAW-associated yield losses in maize are estimated to be between 21 and 53% (Prasanna et al., 2018).

Despite the impact of the FAW on maize yield and quality losses, there have been no dedicated FAW resistance-breeding programs in SSA. This is mainly due to the recent occurrence of the pest in the region, a limited knowledge on the pest's biology and development under local conditions, and a lack of specialized facilities and protocols for FAW rearing and the controlled infestation of test genotypes (Feldmann et al., 2019). Knowledge of a pest's biology, growth and development, and protocols for the artificial rearing of the pest under controlled conditions, create a framework for long term control of the pest using integrated pest management strategies (IPM) (Kasoma et al., 2020a).

Integrated pest management approaches are based on host plant resistance as the most economic and sustainable control strategy (FAO 2018). However, tropically adapted maize germplasm with high levels of FAW resistance have not yet been identified or developed in Africa (Prasanna et al., 2018). In the regions where FAW evolved, including North and South America, standard protocols for the rearing of FAW, inoculation and evaluation of maize genotypes are available, enabling artificial screening, leading to the identification and development of resistant maize cultivars. Several studies have reported laboratory rearing, growth and development of FAW (Revelo and Raun, 1964; Perkins, 1979; Santos et al., 2003; Silva and Parra, 2013; Montezano et al., 2019). Laboratory-reared FAW have been used to screen maize genotypes for resistance breeding (Davis et al., 1989; Williams and Davis, 1990; Isenhour and Wiseman, 1991; Ni et al., 2014).

Artificial screening requires a screenhouse and an insectarium in which to grow sufficient insects to be able to screen maize germplasm reliably (Kasoma et al., 2020a). It also provides the opportunities to gain insights into the biology of the pest through stringent monitoring of the various life-cycle stages. The use of insect populations with a uniform growth stage and a more detailed observation under artificial screening allows for improved understanding of pest-plant interactions (Castro and Pitre, 1988; Williams and Davis, 1990; Santos et al., 2003). Under natural infestation, genotypes may show minimal or no pest damage due to escape rather

than being due to host resistance (Mihn, 1983). Artificial screening ensures that test genotypes are compared under homogeneous test conditions in terms of pest pressure and, therefore, this prevents "escapes."

In SSA, there is a paucity of information on protocols for laboratory rearing of FAW, artificial inoculation and controlled evaluation of genotypes for FAW resistance. There is a need for multi-disciplinary research and standardised methodologies on FAW rearing, inoculation, evaluation and selection of promising genotypes. This will enable the incorporation of host-plant resistance into the management of FAW in SSA. Therefore, the objectives of this study were: 1) To investigate the biology and development of FAW; 2) To develop a standardised methodology for laboratory rearing of FAW; and 3) To evaluate the efficacy of artificial inoculation of FAW using selected maize genotypes and to identify FAW resistant maize genotypes under controlled conditions for resistance breeding.

4.2 Materials and methods

4.2.1 Study site

The study was conducted in an insectarium and in a 35 × 40m screenhouse in the Department of Entomology at the Zambia Agricultural Research Institute (ZARI). The ZARI is located at Mount Makulu Research Station (15°32.87S 28°14.92E, altitude 1225m) in Zambia. The average day light length for the duration of the study was 12:50 h, while the average temperature and relative humidity were 27 ± 1°C and 60 ± 5%, respectively.

4.2.2 Laboratory rearing of FAW

About 30 FAW egg batches and 60 larvae (F0 generation) of mixed instars were collected from unsprayed maize fields from the Zambia Seed Company (ZAMSEED) research farm in Chisamba District Central, Zambia (15022'30.65" S, 28023'22.23" E; Elevation 1251m) using perforated plastic containers. When in the insectarium, the larvae were removed from the containers and transferred singly into Petri dishes (100 x 15 mm, Polystyrene Petri dish, Fischer Scientific, United States) containing either a natural or an artificial diet (Appendix 4.1). The natural diet consisted of tender maize leaves and young stalks from a local, open pollinated variety (OPV) ZM 4342, whereas the artificial diet was prepared from sucrose, soy flour, wheat germ and other ingredients (Appendix 4.2). The eggs were also placed in Petri dishes containing small pieces of fresh and tender maize leaves and left to hatch. After hatching, the neonate larvae were separated into two sets, A and B, for continued rearing on both the natural

and artificial diet. The two sets were maintained separately in fresh Petri dishes in smaller groups of 10-15 larvae per Petri dish. At the third instar, Set A neonates were again separated and placed singly into Petri dishes for continued rearing, along with previously field-collected FAW larvae, which were also maintained singly in Petri dishes. Diet replacements were conducted regularly to ensure a fresh supply of food for the growing larvae through all the instar stages. Between each successive diet change, Petri dishes were cleaned with 5% hypochlorite solution to prevent microbial growth. Set B genotypes were inoculated onto the leaves of potted maize seedlings grown in the screen house using an Artist's paintbrush (Synthetic watercolour brush, Jinjiang Jiaxing Groups Co. Ltd, China) (Appendix 4.3). Care was taken to avoid mixing larvae reared on the natural and artificial diets during both the inoculation and rearing processes to enable independent observations of larvae reared on the two diets. In the case of larvae reared on the natural diet, the maize leaves and stalks were replaced every two to three days, while the artificial diet was replaced with a fresh preparation every four days. To reduce pupal mortality during pupation, the temperature and humidity in the rearing room were adjusted to 26°C and 70 ± 5% relative humidity (RH) using an internal heating system and humidifier, respectively. Paired male and female FAW moths emerging from the pupa were transferred into adult rearing cages fitted with waxy paper to enable mating and subsequent oviposition (Appendix 4.4). The moths were fed on a 5% sugar solution by soaking cotton wool balls in the sugar solution and placing these inside the cages on Petri dish covers. Once egg laying commenced, the fresh eggs were carefully scraped off from the surface of the cages using a clean spatula and placed in new Petri dishes with tender maize leaves, awaiting hatching. Neonate larvae hatching from the eggs (F1) were sorted into Sets A and B at the third instar as before, marking them for continued rearing and inoculation of maize seedlings, respectively. Over 2000 FAW neonates were generated for rearing and inoculation of maize test genotypes.

4.2.3 Artificial screening of maize genotypes with laboratory-reared FAW

4.2.3.1 Plant materials

Sixty-three tropical maize genotypes comprising of 57 CIMMYT inbred lines, four OPVs and two single-cross hybrids as checks were used in the study (Table 4.1). Fifty of the genotypes were selected from previous studies conducted under natural FAW infestation at Mount Makulu Research Station during the 2017-2018 main season and the 2018 off-season cultivation (Kasoma et al., 2020b).

Table 4.1. Maize genotypes evaluated under artificial FAW infestation

Genotype	Type	Source	FAW resistance*	Genotype	Type	Source	FAW resistance *	Genotypes	Type	Source	FAW resistance*
CKDHL0323	Inbred line	CIMMYT	MR	CZL15225	Inbred line	CIMMYT	MR	Teost	OPV	NPGRC	-
CML441-B	Inbred line	CIMMYT	MR	CZL15123	Inbred line	CIMMYT	MR	TL101711	Inbred line	CIMMYT	-
CML488	Inbred line	CIMMYT	MR	CZL15231	Inbred line	CIMMYT	S	TL102562	Inbred line	CIMMYT	-
CML491	Inbred line	CIMMYT	S	CZL15234	Inbred line	CIMMYT	MR	TL116163	Inbred line	CIMMYT	MR
CML538	Inbred line	CIMMYT	MR	CZL16015	Inbred line	CIMMYT	S	TL118367	Inbred line	CIMMYT	-
CML539	Inbred line	CIMMYT	S	CZL16016	Inbred line	CIMMYT	MR	TL12176	Inbred line	CIMMYT	MR
CML545-B	Inbred line	CIMMYT	MR	CZL16080	Inbred line	CIMMYT	S	TL13159	Inbred line	CIMMYT	MR
CML546-B	Inbred line	CIMMYT	S	CZL16084	Inbred line	CIMMYT	MR	TL1316	Inbred line	CIMMYT	MR
CML547-B	Inbred line	CIMMYT	S	CZL16091	Inbred line	CIMMYT	MR	TL139113	Inbred line	CIMMYT	-
CML548-B	Inbred line	CIMMYT	MR	CZL16093	Inbred line	CIMMYT	S	TL139180	Inbred line	CIMMYT	MR
CML572	Inbred line	CIMMYT	MR	CZL16095	Inbred line	CIMMYT	MR	TL142017	Inbred line	CIMMYT	R
CZL03011	Inbred line	CIMMYT	S	CZL16098	Inbred line	CIMMYT	MR	TL142139	Inbred line	CIMMYT	MR
CZL052	Inbred line	CIMMYT	MR	CZL16137	Inbred line	CIMMYT	S	TL142151	Inbred line	CIMMYT	MR
CZL1310c	Inbred line	CIMMYT	MR	CZL16141	Inbred line	CIMMYT	MR	TL14266	Inbred line	CIMMYT	-
CZL1347	Inbred line	CIMMYT	S	EBL1611480	Inbred line	CIMMYT	-	TL145748	Inbred line	CIMMYT	-
CZL1369	Inbred line	CIMMYT	MR	EBL169550	Inbred line	CIMMYT	R	TL1512847	Inbred line	CIMMYT	S
CZL1466	Inbred line	CIMMYT	MR	EBL173782	Inbred line	CIMMYT	-	TL1512845	Inbred line	CIMMYT	MR
CZL15033	Inbred line	CIMMYT	MR	EBL1738809	Inbred line	CIMMYT	-	TL173	Inbred line	CIMMYT	MR
CZL15142	Inbred line	CIMMYT	S	MM501	Hybrid	ZAMSEED	S	VL05120	Inbred line	CIMMYT	-
CZL15209	Inbred line	CIMMYT	MR	MM502	Hybrid	ZAMSEED	MR	ZM4236	OPV	NPGRC	MR
CZL15220	Inbred line	CIMMYT	MR	Pool 16	OPV	ZAMSEED	S	ZM7114	OPV	NPGRC	MR

CIMMYT = International Maize and Wheat Improvement Center; OPV = Open Pollinated Variety; ZAMSEED = Zambia Seed Company; NPGRC = National Plant Genetic Resources Center; - = not rated but selected from a maize seed-increase nursery based on visual observation; R = resistant (a score of 1 to 3); MR = moderately resistant (a score of 4 to 6); S = susceptible (a score of 7 to 10); * FAW reaction based on field evaluation of genotypes by Kasoma et al. (2020) and score equivalence in comparison to Davis et al. (1992)

4.2.3.2 Experimental design and trial establishment

The 63 genotypes were established in 5L capacity plastic pots and evaluated using a randomized complete block design with three replications. Each pot was filled with sandy loam soil, and the soil was mixed with 5g of basal fertilizer (8% nitrogen, 18% phosphorous and 15% potassium) and watered to field capacity. Three seeds per genotype were sown per pot at a depth of 2.5cm. The pots were watered twice a week to ensure sustained moisture for germination. Emerging seedlings were kept free of weeds, and the soil was aerated regularly using a rod to ensure sufficient aeration for the developing roots. Each pot was appropriately labelled for identification.

4.2.3.3 Inoculation of maize genotypes with FAW larvae

The first infestation of the maize genotypes with FAW larvae was conducted 10 days after emergence when the plants were at the three-leaf stage (V3). Five FAW larvae of the second to the third instar were used per plant. An artist's brush was used to transfer the larvae from the petri dish onto the youngest fully-formed leaf for infestation. A second infestation was done at the fourth vegetative growth stage (V4) using six third to fourth instar FAW larvae per plant. This was carried out eight days after the first infestation.

4.2.4 Data collection

4.2.4.1 Laboratory evaluation of FAW growth and development

Data on FAW life-cycle stages, including the duration and description of the salient features distinguishing various growth stages, was collected during the laboratory rearing of the insect. The number of surviving FAW egg batches were counted for larvae reared on the natural and artificial diet. An egg batch was considered as surviving if $\geq$ 30% of the larvae that originally hatched from the egg mass successfully developed to pupa.

4.2.4.2 Seedling evaluation for FAW resistance

Seedling resistance was assessed based on FAW damage scores on seedling plants four days after the first infestation. Following the second infestation, FAW leaf-damage rating was performed at six-day intervals for four weeks. A 1-9 scale adapted from the Davis et al. (1992) for rating FAW damage was used, where a score of 1 represented a healthy plant with no

damage symptoms and 9 represented a completely damaged plant with no possibility of recovery (Table 4.2).

Table 4.2. Rating scale used to score maize genotypes artificially infested with FAW larvae

Symptom	Score
No visible damage	1
2-4 window-pane damaged portions	2
2-4 window-pane damaged portions and 2-4 pin / shot holes	3
5-10 window-pane damaged portions and shot holes	4
10-15 window-pane damaged portions and shreds only	5
10-15 window-pane damaged portions shot holes and shreds	6
10-15 window -pane damaged portions , shot holes, shreds and traces of whorl damage	7
≥ 15 window-pane damaged portions, shot holes, shreds and moderately damaged whorl	8
≥15 widow-pane damaged portions, shot holes, shreds and completely damaged whorl	9

The type, magnitude and frequency of occurrence of FAW damage symptoms on the maize genotypes were described and recorded during rating with the 1-9 scale.

4.2.5 Data analysis

Data on the survival of FAW egg batches was converted to percent egg-batch survival and presented using a bar graph.

The Shapiro-Wilks test of normality was done before analysis of variance on the leaf damage data using GenStat, 18th edition (Payne, 2017). The area under pest progress curve (AUPPC) was calculated for each genotype following Heinrichs and Miller (1991), and Jeger and Viljanen-Rollinson (2001) as follows:

$$AUPPC = \sum_{i=1}^{n=1} \left[\left(\frac{FLD_i + FLD_{i+1}}{2} \right) (t_{i+1} - t_i) \right]$$

Where,

FLD_i represents the mean of the ith FLD across the three replications, beginning with FLD0 to FLD5

Y_{i+1} represents the mean the ith FLD plus 1

t_i represents the ith time point at which leaf damage assessments were made, beginning with 14 days through to 32 days after the first signs of FAW infestation

t_{i+1} represents the ith time point plus 1

The genotypes were classified into top, average and bottom performance genotypes.

4.3 Results

4.3.1 Evaluation of FAW growth and development

The four FAW life-cycle stages, namely egg, larva, pupa and moth, were clearly distinguished during the rearing of FAW in the laboratory (Figure 4.1). The various sub-stages of the life cycle are summarised in Figure 4.2. The different stages in the life cycle of the FAW varied in duration. The larval stage was the longest and lasted up to 17 days. The egg, pupa and moth lasted 4, 11 and 12 days, respectively. The larval stage was divided into different instar stages. Within the larval stage, the third instar was the shortest, followed by the second and fourth instars, which had similar duration. Important transitional phases related to the egg, larva and pupal stages were also identified (Figure 4.2). The observed transitional phases included the progressive egg-colour changes, the blackhead, ecdysis and pre-pupal phases, which depicted early and late transitions related to the egg, larval and pupal stages.

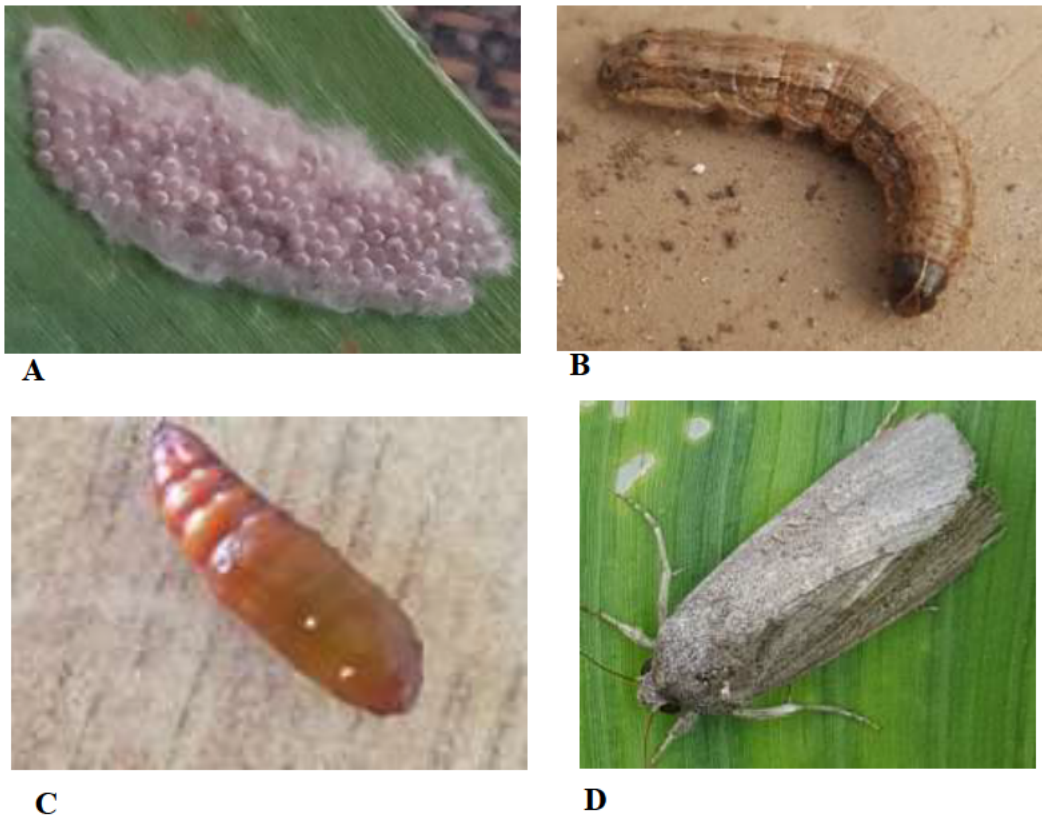


Figure 4.1. Life cycle stages of the FAW: Note **A**: egg-mass, eggs hatch within 2-4 days after they being laid. **B**: larva, feeding stage during which the FAW destroys crops. **C**: pupa, transitory dormant stage. **D**: Adult female FAW, less conspicuous than male moth
Note: The life cycle completes within 30 - 40 and 32 - 44 days for the male and female FAW, respectively

Stage	Sub-stage	Number of days lapsed for the stage/sub stage	Description	Image
Egg	I	<1	Freshly laid eggs covered by scales. Eggs appear green-grey for 12 hours before they darken	
Egg	II	1	Cream-white to pink colour transitioning into a brown colour	
Egg	III	1	Darkening egg mass as it approaches hatching. Before hatching, the egg mass appears grey-black	
Egg-larva	Eclosion	<1	Larval black heads visible through egg mass as they emerge from the egg shells	
Larva	Blackhead	<1	Newly hatched larvae on tender maize stalk after devouring the remains of the eggs from which they hatched and remaining dormant for about 5 to 6 hours	

Larva	I	2 - 2.5	1st instar larvae on a maize leaf. The body appears whitish with a shiny blackhead. Young larvae exhibit positive phototropism and snuggle in a position directly to the sun.	
Larva	II	1.5 – 2	2nd instar larva on a maize leaf with a cream to pale white body and blackhead prior to initiation of feeding. on maize leaf	
Larva	III	1	3rd instar larva, the body begins to darken to a light brown and green colour after the first feeding on maize leaves	
Larva	IV	1.5 – 2	4th instar larva, the body further darkens with more feeding. Body markings becoming more prominent	
Larva	V	2 – 3	5th instar larva, body assuming a brown appearance and body markings become more visibly defined including the inverted Y on the head and the trapezoidal black dots in the second last segment	
Larva	VI	3 – 4	6th instar larva on a maize tassel and surrounded by frass, larva has a greyish brown appearance fully defined segments, body and head markings	

Larva	Ecdysis/ malting	1 – 2	Larva sheds its outer cuticular skeleton between instars leaving a colourless patch in neck area. Visible ecdysis observed to last approximately 12 to 28 hours	
Larva	Ecdysis/ malting	1 – 2	The outer malted FAW skeleton observed in rearing container. It appears black and has the exact shape of the larva from which it was ecdysed	
Larva-pupa	Pre-pupa	2 – 3	The adult FAW larva stops feeding and shortens its length as it prepares for pupation. Its body segments become clearly defined ridges, and the body markings appear closer to each other	
Pupa	-	8 – 9	The shortened pre-pupal FAW begins to form an oval-shaped cocoon using leaf particles. The process culminates into a full-fledged pupa. The cocoon gradually changes from green-pink to orange-brown	

Figure 4.2. Detailed descriptions of the sub-stages of the life cycle of FAW

4.3.1.1 Survival rate of FAW on natural and artificial diets

The survival rate was higher for the FAW larvae raised on the natural diet than the artificial diet. Out of 15 FAW egg batches reared on the natural diet, which all hatched into neonate larvae, 12 batches survived and grew through the larval instars and successfully pupated. For

larvae reared on the artificial diet, six out of 15 collected FAW egg batches survived and grew through the larval instars to pupation (Figure 4.3).

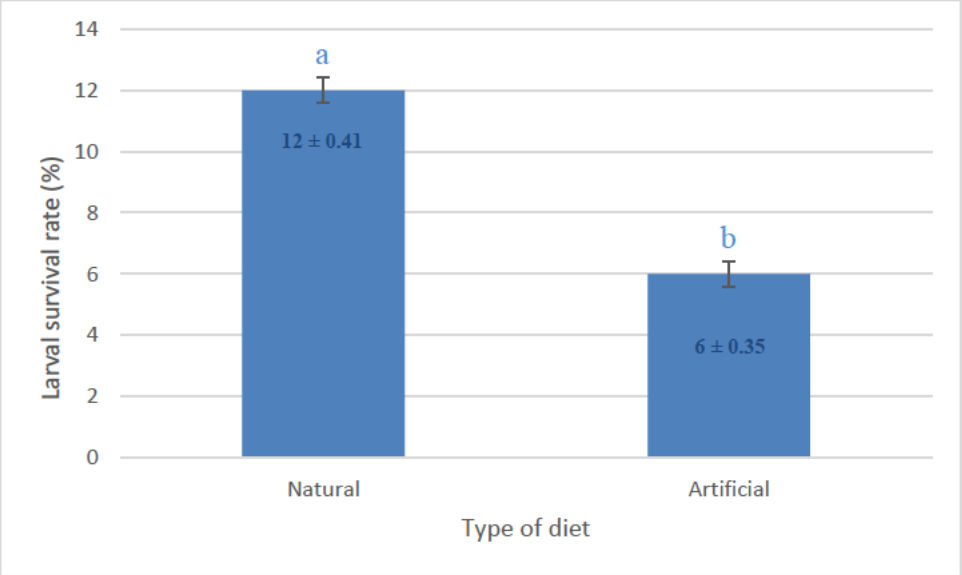


Figure 4.3. Rate of survival of FAW larva reared on the natural and artificial diet.
Note: letters a and b denote significant differences.

4.3.1.2 Response of maize genotypes to FAW larvae infestation

Analysis of variance revealed non-significant differences among the test genotypes for the first leaf damage score when infested with FAW larvae (Table 4.3). Differences among the genotypes were significant ($p < 0.05$) for the second leaf damage score and highly significant ($p < 0.01$) for the third, fourth and fifth leaf scoring. There were non-significant differences in FAW leaf damage caused by the FAW whether they were raised on artificial or natural diets.

Table 4.3. Analysis of variance for FAW leaf damage among 63 genotypes evaluated under artificial FAW infestation in the screen house

Source	df	FLD1	FLD2	FLD3	FLD4	FLD5
Replication	2	0.30^{ns}	1.60^{ns}	5.75^{ns}	8.83^{ns}	5.06^{ns}
Diet	1	0.20^{ns}	0.28^{ns}	0.00^{ns}	0.04^{ns}	0.086^{ns}
Genotype	62	0.96	6.33*	3.40**	1.51**	1.40**
Genotype × diet	62	0.06^{ns}	0.03^{ns}	0.09^{ns}	0.03^{ns}	0.13^{ns}
Residual	189	0.86	4.39	2.19	0.97	0.87

DF = Degrees of freedom; ns-not significant; *,** = significant at $p < 0$ 05 and $p<0.01$ respectively; ns = non-significant; FLD1 = refers to the first FAW leaf damage rate recorded at 4 days after the first infestation; FLD2, FLD3, FLD4 and FLD5 = refer to the first, second, third, fourth and fifth FAW leaf-damage rates recorded at six-day intervals after the second infestation

4.3.1.3 Mean performance of test genotypes and area under pest progress curve (AUPPC)

Most test genotypes had FLD1 ratings below the score of 2. Only 6% genotypes had an FLD1 score of 3, while 30% had a score of 2 at FLD1. FAW damage scores for the genotypes were most variable at FLD3 followed by FLD4 (Table 4.4). The AUPPC values for the genotypes ranged from 61.63 to 137.94, with the genotypes TL02562 and CZL1347 having the lowest and highest values, respectively. The mean performance for all 63 genotypes is presented in Appendix 4.5.

Table 4.4. Mean performance of the top, middle and bottom five maize genotypes arranged according to AUPPC values when evaluated under artificial FAW infestation

Name	FLD0	FLD1	FLD2	FLD3	FLD4	FLD5	AUPPC
Top five performing genotypes							
TL02562	0.00	0.86	0.87	2.17	4.81	4.86	61.63
TL142151	0.00	1.83	1.61	2.37	4.81	4.86	67.29
TL12176	0.00	1.08	1.21	3.61	4.12	4.59	67.39
TL13159	0.00	2.33	1.25	1.83	5.83	5.83	70.98
Teost	0.00	1.33	1.75	2.00	5.50	5.67	72.50
Middle five performing genotypes							
EBL169550	0.00	1.50	1.50	3.17	6.17	6.83	85.52
Pool 16	0.00	1.33	2.09	4.29	4.60	6.82	86.33
CML545-B	0.00	2.33	2.33	3.32	5.82	5.85	86.39
CZL16015	0.00	1.75	2.33	3.17	5.83	6.33	87.00
TL1316	0.00	2.33	2.33	3.50	5.83	5.83	87.48
Bottom five performing genotypes							
CML547-B	0.00	2.91	2.84	4.00	7.00	7.33	105.04
CZL16141	0.00	1.92	1.72	5.78	6.76	6.82	106.04
CZL15225	0.00	3.24	3.70	5.00	6.67	6.67	112.20
CZL15220	0.00	1.83	2.33	5.32	7.82	7.85	116.39
CZL1347	0.00	3.05	5.43	6.33	7.33	7.80	137.94
Statistics							
GM	0.00	1.85	2.05	3.26	6.05	6.33	87.33
CV (%)	-	49.60	92.60	45.60	16.30	14.80	-
LSD (0.05)	-	1.50	3.40	2.40	1.60	1.51	-
SE	-	0.93	2.10	1.48	0.98	0.93	-

FLD0 = stage prior to FLD1 with no visible FAW damage symptoms; FLD1 = refers to the first FAW leaf damage rate recorded at 4 days after the first infestation; FLD2, FLD3, FLD4, FLD5 = refer to the first, second, third, fourth and fifth FAW leaf-damage rates recorded at six-day intervals after the second infestation; AUPPC = Area Under Pest Progress Curve GM = grand mean; CV = coefficient of variation; LSD = least significant difference; SE = standard error

FAW leaf damage ratings of the test genotypes gradually increased from 0.00 to 6.33 between FLD0 to FLD5 (Figure 4.4). A relatively rapid increase in the FAW damage ratings was observed between FLD0 and FLD1 and between FLD3 and FLD4.

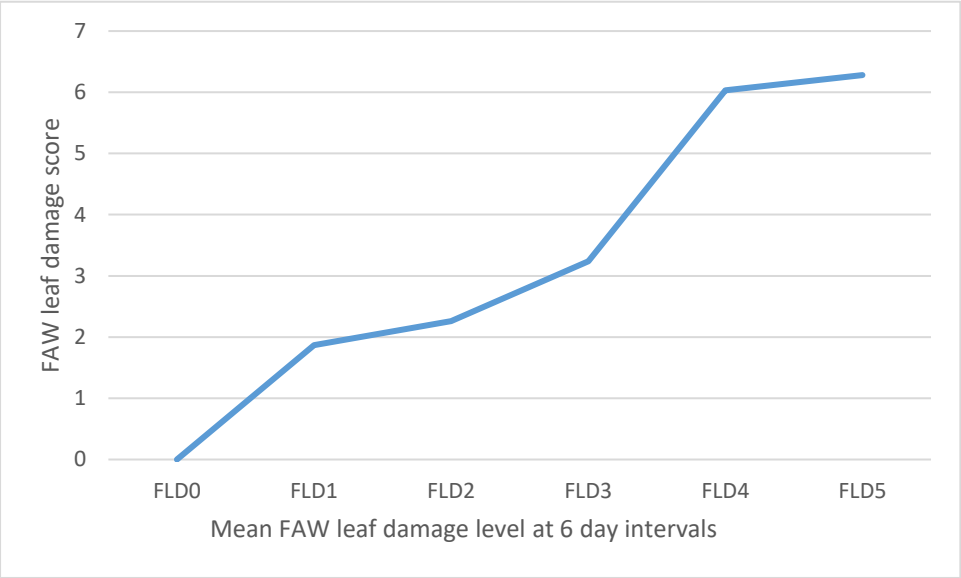
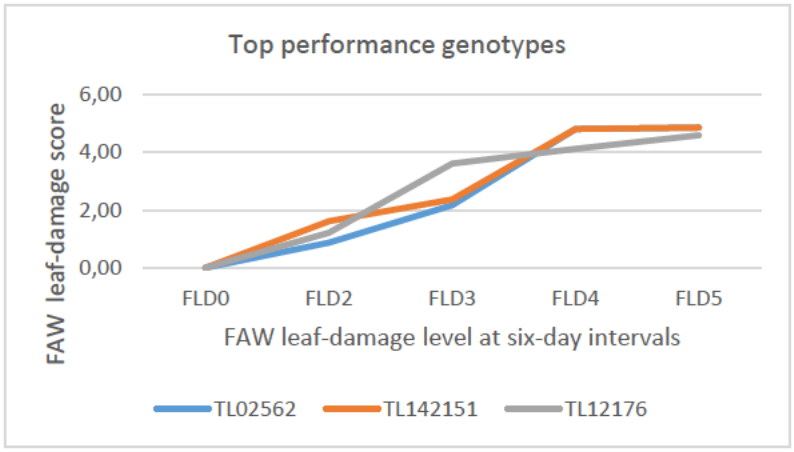
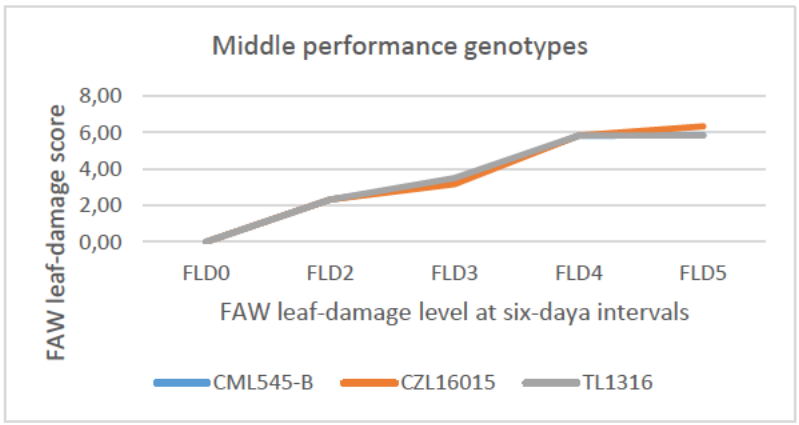


Figure 4.4. Nature of FAW damage progression on maize genotypes from FLD 0 to FLD5

The top, middle and bottom performing genotypes showed clear damage progression trends, which increased from FLD0 to FLD4 and levelled off at FLD5 (Figure 4.5). The final leaf damage scores for the top, middle and bottom performing genotypes were 4.86, 6.33 and 7.80, respectively.



A



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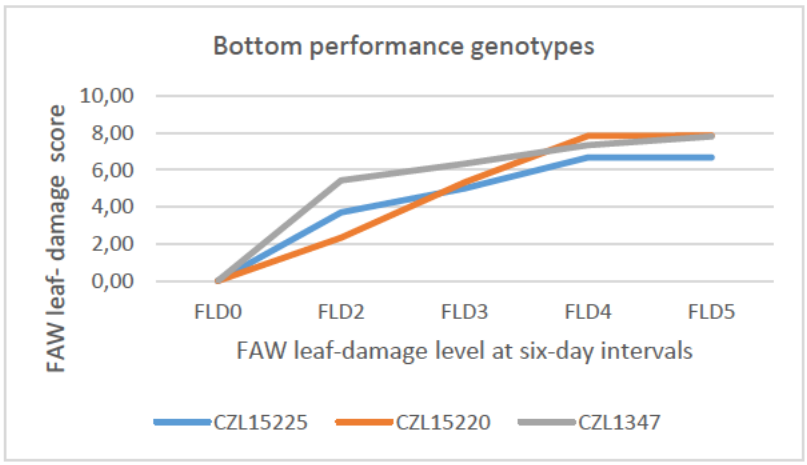

C

Figure 4.5. Pattern of damage progression for A- top performance, B- middle-performance and C- bottom performance maize genotypes evaluated under artificial FAW infestation

4.3.1.4 Nature of FAW damage and reaction of test genotypes to artificial FAW infestation

The test genotypes showed a set of damage characteristics in response to artificial infestation with FAW larvae. Damage characteristics ranged from 0 (no signs of FAW feeding) to 5 (visible leaf and whorl damage). The nature of the specific FAW-related damage observed in 15 selected genotypes is shown in Table 4.5. FAW-related damage profiles of all the 63 test genotypes is presented in Appendix 4.6. Damage to the leaf only was the most common FAW-related symptom among the genotypes, while damage to the leaf and whorl was the next most common symptom of FAW damage. Stalk damage was the least common damage symptom observed among the genotypes.

Table 4.5. FAW damage type and magnitude revealed by 15 representative genotypes evaluated under artificial FAW infestation

	Damage type						
Genotype	None	Whorl only	Leaf only	Stalk	Leaf/whorl and fresh frass	Leaf and whorl	Number of plants at final assessment
CML545-B	0	0	5	0	0	1	6
CZL1466	0	2	0	0	1	1	4
CML491	0	0	3	0	0	0	3
CZL0310c	0	0	2	0	0	3	5
CML539	0	0	5	1	0	0	6
CZL15142	0	0	0	0	0	2	2
VL050120	0	0	1	0	0	0	1
CZL16095	0	0	1	0	0	1	2
ZM4236	0	0	0	0	0	3	3
EBL1611480	0	1	4	0	0	0	5
EBL169550	0	0	0	0	1	5	6
EBL173782	1	0	1	0	1	0	3
MM501	0	0	3	0	1	1	5
Pool 16	1	0	1	0	0	1	3
TL142151	0	0	1	0	0	0	1
Total number of plants showing the damage*	3	7	101	1	14	80	206

* refers to the total across all the 63 genotypes and each genotype was represented by six plants across three replications (two plants per replication)

4.4 Discussion

4.4.1 Laboratory rearing of FAW

The present study successfully reared FAW in Petri dishes using the egg and larval stages and in oviposition cages using adult moths. Although the laboratory conditions used in this study enabled successful observation of all the life-cycle stages, a few deviations from what is currently known about FAW development in nature were observed. For instance, oviposited eggs were mostly heaped rather than layered as occurs naturally. Also, most of the cocoons appeared more fragile compared to those collected from maize fields. The heaping of eggs in the laboratory may be attributed to the effect of confinement of the female moths within the rearing cages. The fragile appearance could be associated with the absence of soil particles typically used by the insect for developing the pupal casing under field conditions (Luginbill, 1928).

The observed length of the entire FAW life-cycle in this study (Figure 4.1) and the duration of individual life-cycle stages under the experimental conditions used corroborated with previous studies (Castro and Pitre, 1988; Hardke et al., 2015). Although most freshly laid eggs collected from maize fields hatched within an average of three days, a few egg batches did not hatch at all, probably due to unfavourable temperatures, parasitism or the egg masses trapped neonates that failed to emerge successfully, due to injury (Luginbill, 1928; Du Plessis et al., 2020). Egg masses gave rise to variable numbers of neonates that actively dispersed whenever the Petri dish was opened, by means of cobweb-like silk threads after a period of dormancy. In addition to dispersal, neonates and young larvae of the first and second instars are known to use the silk threads as a defence mechanism that helps them to drop rapidly to the ground whenever they are threatened (Baur and Yeargan, 1994).

The larval period, the longest of the life-cycle stages, is the feeding stage of the FAW, within which temperature and diet are very important factors for growth and development. López et al. (2019) reported that FAW larvae required minimum temperatures ranging between 8 and 10°C for survival in Mexico. In South Africa, the minimum temperature for FAW larval survival was reported to be 12°C (Du Plessis et al., 2020). Santos et al. (2003) observed rapid FAW larval growth and shorter instars under higher temperatures. Previous studies suggest the use of heat units or growth degree day units (GDDU) as a reliable means of determining the developmental rate of FAW (López et al., 2019; Du Plessis et al., 2020). Increased larval survival on the natural diet compared to the artificial diet corroborates to the observation by Castro and Pitre (1988), who recorded high larval mortality on soybean, one of the key

ingredients of the artificial diet used in this study. Thus, a diet composed largely of maize extracts would be more suitable for raising the FAW in an insectarium. Feeding during the larval stage progressively increases, with the first three instars observed to only skeletonize and punch small holes through maize leaves, while the fourth to sixth instars are responsible for more severe damage, including eating whole-leaf portions and destroying small plants. In FAW monitoring and surveillance, farmers should monitor their fields for skeletonized maize leaves, which would indicate the presence of FAW because very few lepidopteran species cause such a damage (Luginbill, 1928; Prasanna et al., 2018). The FAW larva is a gregarious feeder during its last instar stage, usually the sixth instar. Notably, a seventh instar sometimes occurs. It is not known precisely what leads to the occurrence of a seventh instar but the diet and environmental conditions may contribute. Instar stage changes can be determined by careful monitoring of body colour, body length and head capsule widths. The transition from one instar to the next is marked by ecdysis or moulting, a process through which the larva sheds its outer layer. Ecdysis would be a single, reliable means of identifying the change from one instar to the next except that the shedding of the outer skeleton is not easily seen in earlier instars because of their small size. In addition, ecdysis has implications for FAW control, considering that moulted larvae are powerless and relatively easy to destroy with pesticides or biocontrol agents.

Following the instar stage of rapacious feeding, the FAW larva moves into a slothful pre-pupal phase when it discontinues feeding (Chapman, 1999). In nature, the pre-pupal phase is not commonly observed because it occurs in the soil and lasts only for a maximum of three days under warm conditions. Laboratory rearing of FAW offers the opportunity to view the pre-pupal phase and to observe its features before the actual pupation occurs. Although the duration of pupation observed in this study was 8 to 9 days, previous studies have reported a maximum pupation period of 45 days (Luginbill, 1928). Under field conditions, pupation mostly occurs underground, when pre-pupal larvae burrow in the soil to a depth of 25 to 75mm. In this study, pupation experienced the highest mortality, with the pupal casing of dead pupae assuming a brown to black appearance.

The genders of adult FAW moths were easily distinguishable by their colour patterns, with female moths being comparatively dull coloured compared to the male moths (Luginbill, 1928; Capinera, 1999). Being nocturnal, the FAW were dormant during the day and only moved when agitated. They were observed to feed and lay eggs late at night. This is in agreement with reports by Luginbill (1928), who found that laboratory-reared FAW moths laid eggs after mid-

night. Female moths generally outlived the male moths, although both were observed to lose vitality progressively. Successful mating and oviposition were observed in the rearing cages, but counts of egg batches were not conducted because of the limited number of surviving moths following high levels of mortality at pupation.

4.4.2 Artificial infestation of maize genotypes

The lack of differences in leaf damage caused by FAW raised on the two different diets shows that the larva are equally damaging on maize despite their previous diet. This could be attributed to the pest's rapid regeneration ability and its preference for maize leaves. Maize is the preferred host for FAW and the larva quickly adapted to the availability of the preferred feed. Therefore, the diet only affects the mortality or survival rate of the FAW larvae but does not reduce efficacy, suggesting that a natural diet made up of maize extracts would be more suitable.

The non-significant differences exhibited by the test genotypes for the first FAW leaf damage assessment (FLD) could be attributed to the uniform nature of feeding damage caused by first to third instar larvae that were inoculated on the maize plants. Leaf-damage assessments during the FLD3 to FLD5 revealed significant differences among the test genotypes. This may be because the assessments were conducted at a time when the inoculated larvae had developed into differentially advanced instars in response to the differences among the host maize genotypes. In addition, later assessments were conducted when the host-pest interaction was sufficiently established to trigger the inherent plant-defence mechanisms whose intensity would depend on the genetic background of the host plant (Badji et al., 2020). The most variable leaf-damage ratings showed up at FLD3, suggesting that the best time to detect differences in FAW leaf damage among the test genotypes under the given experimental conditions was at three weeks after the first infestation. However, to capture differences more accurately, repeated assessments during the vegetative growth are recommended. The levelling off of the damage curve at FLD5 (Figure 4.5) may be attributed to the pest's known characteristic of abandoning foliar damage to strategically position itself to attack emerging ear shoots and tassels that become visible with magnification by V6 (Mueller and Sisson, 2013; Prasanna et al., 2018). A more detailed investigation of the profiled genotypes and similar germplasm is required to enhance our understanding of maize responses to FAW feeding.

The defined set of damage characteristic in response to FAW-feeding has useful implications for pest management. Farmers are encouraged to closely monitor their fields for pest damage

symptoms, which constitute many of the symptoms identified in this study. The high frequency of leaf and whorl damage symptoms observed in this study agrees with the findings of Abrahams et al. (2017), who reported that foliar damage is the most typical FAW-related damage symptom in maize.

The damage profiles of the test genotypes enabled the identification of promising genotypes in terms of damage severity for further breeding. The genotypes TL13159, TL02562, TL142151, VL050120 and CML548-B were strongly FAW resistant, while CML545-B, CZL1310c, CZL16095, EBL169550, ZM4236 and Pool 16 were moderately resistant to FAW, and were selected for breeding. FAW preferences for these genotypes were low (Pool 16), moderate (TL13159, TL142151, CML548-B, CML545-B, CZL1310c, CZL16095, ZM4236) and high (EBL169550) when previously screened under natural FAW infestation by Kasoma et al. (2020).

In conclusion, this study determined the salient features of FAW growth and development under local conditions using natural and artificial diets. This will assist growers to implement integrated FAW management strategies, and will assist plant breeders to undertake controlled host-plant resistance breeding. Furthermore, the study established a standardised laboratory- and screenhouse-based protocol for mass rearing and artificial infestation of FAW to screen maize genotypes for resistance breeding programs in Zambia or other SSA Africa countries.

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4.6 Appendices

Appendix 4.6.1. Diets used for rearing of FAW on Petri dishes. A- Natural diet of maize leaves and stalks. B- Artificial diet containing wheat, soy and other ingredients

Appendix 4.6.2. Artificial diet used for laboratory rearing of FAW

Ingredient	Quantity	Apparatus	Ingredients of dry mix
Agar	28.5g	Microwave	Soy flour
Dry mix	216g	Blender	Sucrose (50%)
Antibiotic	1.5g	Spatula	Wheat germ
	Procedure		Salt mix
Step 1: Add agar to 875ml of water			USDA vitamin pre mix
Step 2: Bring to boil for 1 minute			Fibre
Step 3: Transfer agar solution to blender containing dry mix			Methyl parabene, Sorbic acid
Step 4: Blend for 30 seconds			Ascorbic acid
Step 5: Dispense immediately			
Step 6: Store diet in refrigerator			

USDA = United States Department of Agriculture

A **B**

Appendix 4.6.3. Screening of maize genotypes using laboratory reared FAW larvae. A- Artificial inoculation using an artist's paintbrush with third instar FAW larvae. B- Visual observation of inoculated maize plants prior to damage rating

Appendix 4.6.4. Rearing cage for adult FAW moths

Appendix 4.6.5. Mean performance and AUPPC values of 63 tropical maize genotypes when evaluated under artificial FAW infestation

Name	FLD0	FLD1	FLD2	FLD3	FLD4	FLD5	AUPPC
TL02562	0.00	0.86	0.87	2.17	4.81	4.86	61.63
TL142151	0.00	1.83	1.61	2.37	4.81	4.86	67.29
TL12176	0.00	1.08	1.21	3.61	4.12	4.59	67.39
TL13159	0.00	2.33	1.25	1.83	5.83	5.83	70.98
Teost	0.00	1.33	1.75	2.00	5.50	5.67	72.50
CML548-B	0.00	1.25	1.25	2.00	5.67	6.67	73.50
TL14266	0.00	2.08	1.83	2.00	5.67	5.67	73.98
CZL15231	0.00	1.83	2.33	3.17	4.33	5.17	74.50
CZL052	0.00	1.33	1.33	2.50	5.83	5.83	75.48
CZL1369	0.00	1.10	1.22	2.83	5.78	5.84	76.52
CZL15234	0.00	1.83	2.00	2.33	5.67	5.67	76.98
CML539	0.00	2.17	2.50	2.83	5.00	5.17	77.48
CZL16137	0.00	1.58	1.92	2.00	6.00	6.00	77.52
VL050120	0.00	1.92	1.33	3.17	5.33	6.33	77.99
TL151845	0.00	1.33	1.67	1.67	6.33	6.67	78.04
TL139113	0.00	1.67	2.33	2.33	5.67	5.67	78.96
MM502	0.00	1.97	1.58	2.86	5.83	5.83	79.14
ZM7114	0.00	1.87	2.42	2.58	5.33	5.83	79.50
CZL16080	0.00	1.50	1.50	2.00	6.50	6.50	79.50
EBL1611480	0.00	1.42	1.42	2.83	6.00	6.00	79.50
TL142017	0.00	1.83	1.83	2.67	6.00	6.00	81.00
TL139180	0.00	1.83	2.17	2.67	5.67	6.00	81.04
CZL16084	0.00	1.75	2.33	2.36	5.88	6.37	82.54
CZL15209	0.00	2.25	2.67	2.67	5.67	5.67	83.04
CZL16093	0.00	1.58	1.83	2.83	6.17	6.17	83.46
CML491	0.00	3.02	2.87	3.11	5.31	5.36	83.78

Appendix 4.6.5 Continued

TL118367	0.00	1.29	1.50	3.00	6.50	6.50	85.50
TL101711	0.00	0.78	2.17	2.83	6.17	6.17	85.50
EBL169550	0.00	1.50	1.50	3.17	6.17	6.83	85.52
Pool 16	0.00	1.33	2.09	4.29	4.60	6.82	86.33
CML545-B	0.00	2.33	2.33	3.32	5.82	5.85	86.39
CZL16015	0.00	1.75	2.33	3.17	5.83	6.33	87.00
TL1316	0.00	2.33	2.33	3.50	5.83	5.83	87.48
TL173	0.00	1.25	1.33	3.50	6.50	6.50	87.48
CML488	0.00	2.16	2.59	3.23	5.84	5.87	87.56
CZL16095	0.00	1.50	2.00	2.33	6.67	7.33	87.98
CZL16098	0.00	0.83	0.83	3.50	6.67	7.33	87.98
CZL16091	0.00	1.54	1.46	2.61	7.09	7.12	88.31
CML546-B	0.00	2.02	1.86	3.37	5.80	7.85	89.75
EBL173783	0.00	1.50	1.75	3.50	6.50	6.50	90.00
TL116163	0.00	1.50	2.67	3.33	6.00	6.00	90.00
CML572	0.00	2.27	2.37	2.61	6.62	6.85	90.14
CZL1310c	0.00	0.99	0.95	3.57	7.07	7.10	90.86
CKDHL0323	0.00	2.67	2.67	4.00	5.67	5.88	91.67
CZL1466	0.00	1.67	1.67	2.67	7.33	7.33	92.04
CML538	0.00	0.99	0.95	4.17	6.83	6.83	92.22
EBL1738809	0.00	2.33	2.83	2.83	6.50	6.50	92.46
CZL1523	0.00	2.04	1.96	3.61	6.59	6.62	92.81
ZM4236	0.00	2.08	2.83	3.83	5.83	6.33	93.95
CZL16016	0.00	2.25	2.75	3.58	6.17	6.33	93.98
TL145748	0.00	2.33	2.08	3.50	6.83	6.83	94.98
CZL15142	0.00	2.67	2.75	4.17	5.83	6.17	95.02
CZL15033	0.00	3.05	2.81	3.84	6.33	6.43	97.15
TL1412139	0.00	1.53	2.58	4.58	5.83	6.50	97.46
TL1512847	0.00	1.33	2.75	4.50	5.83	6.50	98.00
MM501	0.00	1.92	1.58	4.17	7.17	7.17	99.00
CZL03011	0.00	2.50	3.00	4.33	6.33	6.67	101.98
CML441-B	0.00	2.50	3.42	4.67	5.83	7.00	104.54
CML547-B	0.00	2.91	2.84	4.00	7.00	7.33	105.04
CZL16141	0.00	1.92	1.72	5.78	6.76	6.82	106.04
CZL15225	0.00	3.24	3.70	5.00	6.67	6.67	112.20
CZL15220	0.00	1.83	2.33	5.32	7.82	7.85	116.39
CZL1347	0.00	3.05	5.43	6.33	7.33	7.80	137.94
GM	0.00	1.85	2.09	3.26	6.05	6.33	87.33
CV (%)	-	49.60	92.60	45.60	16.30	14.80	-
LSD (0.05)	-	1.50	3.40	2.40	1.60	1.51	-
SE	-	0.93	2.10	1.48	0.98	0.93	-

FLD0 = stage prior to FLD1 with no visible FAW damage symptoms; FLD1 = refers to the first FAW leaf damage rate recorded at 4 days after the first infestation; FLD2, FLD3, FLD4, FLD5 = refer to the first, second, third, fourth and fifth FAW leaf-damage rates recorded at six-day intervals after the second infestation; AUPPC = Area Under Pest Progress Curve GM = grand mean; CV = coefficient of variation; LSD = least significant difference; SE = standard error

Appendix 4.6.6. Nature and magnitude of FAW damage revealed by 63 tropical maize genotypes evaluated under artificial FAW infestation

	Damage characteristic						
Genotype	None	Whorl only	Leaf only	Stalk	Leaf/whorl and fresh frass	Leaf and whorl	Number of plants at final assessment
CKDHL032	0	0	1	0	0	5	6
CML441-B	0	2	0	0	0	2	4
CML488	0	0	3	0	0	0	3
CML491	0	0	1	0	0	1	2
CML538	0	0	0	0	0	2	2
CML539	0	0	5	1	0	0	6
CML545-B	0	0	5	0	0	1	6
CML546-B	0	0	1	0	0	1	2
CML547-B	0	0	1	0	1	0	2
CML547B	0	0	2	0	0	0	2
CML548-B	0	0	0	0	0	2	2
CML572	0	0	1	0	0	0	1
CZL03011	0	0	4	0	0	1	5
CZL052	0	0	4	0	0	0	4
CZL1310c	0	0	2	0	0	3	5
CZL1347	0	0	1	0	0	2	3
CZL1466	0	2	0	0	1	1	4
CZL15033	0	0	1	0	2	0	3
CZL15142	0	0	0	0	0	2	2
CZL15209	0	0	3	0	0	2	5
CZL15220	0	0	1	0	0	2	3
CZL15225	0	0	3	0	1	1	5
CZL1523	0	0	0	0	1	1	2
CZL15231	0	1	0	0	0	2	3
CZL15234	0	0	5	0	0	1	6
CZL16015	0	0	2	0	1	1	4
CZL16016	0	0	2	0	1	2	5
CZL16080	0	0	2	0	1	0	3
CZL16084	0	0	0	0	1	1	2
CZL16091	0	0	3	0	0	0	3
CZL16093	0	0	3	0	0	0	3
CZL16095	0	0	1	0	0	1	2
CZL16098	0	0	1	0	0	2	3
CZL16137	0	0	5	0	0	0	5
CZL16141	0	0	0	0	0	2	2
EBL1611480	0	1	4	0	0	0	5
EBL169550	0	0	0	0	1	5	6
EBL173782	1	0	1	0	1	0	3
EBL1738809	0	0	2	0	0	2	4
MM501	0	0	3	0	1	1	5
MM502	0	0	0	0	0	1	1
Pool16	1	0	1	0	0	1	3
Teost	0	0	1	0	1	1	3

Appendix 4.6.6 Continued

TL101711	0	0	1	0	0	0	1
TL102562	0	0	1	0	0	0	1
TL116163	0	1	2	0	0	0	3
TL118367	0	0	1	0	0	1	2
TL13159	0	0	0	0	0	2	2
TL1316	0	0	3	0	0	2	5
TL139113	0	0	1	0	0	1	2
TL139180	0	0	1	0	0	3	4
TL142017	0	0	1	0	0	2	3
TL142139	0	0	2	0	0	4	6
TL142151	0	0	1	0	0	0	1
TL14266	0	0	3	0	0	2	5
TL145748	0	0	4	0	0	2	6
TL151284	0	0	0	0	0	1	1
TL151845	1	0	1	0	0	1	3
TL173	0	0	2	0	0	4	6
VL05120	0	0	1	0	0	0	1
ZM4236	0	0	0	0	0	3	3
ZM7114	0	0	1	0	0	0	1
Total number of plants showing the damage	3	7	101	1	14	80	206

CHAPTER 5. REVEALING THE GENETIC DIVERSITY OF MAIZE POPULATIONS BY PHENOTYPIC TRAITS AND DArT-SEQ MARKERS FOR VARIABLE RESISTANCE TO FALL ARMYWORM

Abstract

The fall armyworm (FAW) is a gregarious insect pest causing substantial yield losses and crop failures in maize and related cereal crops in sub-Saharan Africa due to a lack of resistant varieties and integrated control options. Genetic variation for economic traits including resistance to the FAW damage is a prerequisite in maize improvement programs. The objective of this study was to determine the genetic diversity of 59 maize genotypes of diverse genetic background with variable resistance to fall armyworm, using phenotypic traits and SNP-based DArT markers. The test genotypes were profiled using agro-morphological traits, FAW damage parameters, and Diversity Array Technology Sequencing (DArTseq)-derived single nucleotide polymorphism (SNP) markers. Significant ($p < 0.001$) differences were observed among the genotypes for 13 phenotypic traits with phenotypic coefficient of variation ranging from 2.19 to 51.79%. Notable phenotypic variation was observed for ear position, grain yield, FAW induced leaf and cob damage. The mean gene diversity and polymorphic information content were 0.29 and 0.23, respectively, reflecting a moderate level of genetic variation among the test genotypes when assessed using SNP markers. Analysis of the molecular variance revealed greater genetic variance within a population rather than between populations. Population structure and cluster analysis grouped the test populations into two main clusters. The following genetically divergent open pollinated varieties were selected with favourable agronomic performance and FAW resistance for population improvement or hybrid breeding: Pool 16, ZM 4236 and ZM 7114. The genetic diversity detected within and among the tested populations will facilitate the breeding of maize varieties incorporating farmer-preferred agronomic traits and FAW resistance in Zambia and related agro-ecologies.

Keywords: Fall armyworm, genetic diversity, landrace populations, resistance breeding, single nucleotide polymorphism[3]

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5.1 Introduction

Maize (*Zea mays* L., 2n = 2x = 20) is an important cereal crop cultivated across a wide range of agro-ecologies and cropping systems globally. It is a major staple food accounting for more than 30% of the total caloric intake for over 300 million people in sub-Sahara Africa (SSA) (McCann 2005). The United States of America, Brazil and China are the world's largest maize producers with estimated annual production levels of 370, 259 and 97 million tonnes, respectively (FAOSTAT 2017). In Africa, Nigeria, South Africa, Egypt and Ethiopia are the topmost producers, although maize productivity is relatively low (< 2.1 t ha^{-1}) in the region (OECD 2018). Maize yields in sub-Saharan Africa are variable with mean yields ranging between 0.9 and 1.5 t ha^{-1} (Mulungu and Ng'ombe 2019).

The low maize productivity in sub-Sahara Africa is attributable to various biotic and abiotic stresses and socio-economic constraints (Sharma and Misra 2011). The fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith) emerged in Africa in 2016, and is now the main biotic constraint of maize in SSA (Georgen et al. 2016). The pest has spread across different countries in Africa and Asia over the last three years, threatening food security and livelihoods (Chhetri and Acharya 2019). Hruska and Gould (1997) reported yield losses of up to 43% in maize, depending on pest pressure and environmental conditions. Under severe FAW infestations, yield losses above 50%, or total crop failure are common (Hruska 2019).

The major FAW control strategies include the use of crop protection chemicals, biological control agents, cultural practices and host plant resistance (Prasanna et al. 2018). Several countries in continental America have registered synthetic pesticides with varying modes of action (Abrahams et al. 2017). However, many of these chemicals have become increasingly ineffective due to pesticide resistance (Fatoretto et al. 2017). Furthermore, insecticides are too expensive for most smallholder farmers, and may have negative environmental effects. Biocontrol methods such as parasitoids, entomopathogenic fungi and nucleopolyhedroviruses have reportedly shown promise in controlling FAW (FAO 2018). However, the efficacy of biological control strategies is yet to be evaluated in SSA, where biocontrol options are not readily available in most countries (Prasanna et al. 2018). Cultural practices are unlikely to provide adequate FAW control when used in isolation. Targeted research is required to determine the effectiveness of cultural control methods to control FAW in SSA, given the complex farming systems and variable agro-ecosystems in the region (Abrahams et al. 2017). Banerjee et al. (2017) reported that genetically modified (GM) maize possessing the Cry1F protein derived from *Bacillus thuringiensis* was toxic to lepidopteran insect pests, including

FAW, in Brazil. Nevertheless, FAW appears to have developed resistance to certain GM maize possessing the toxic proteins Cry1F, Cry1Ab and Cry1Ac (Ingber et al. 2017), opening up further debate on whether to adopt GM technology in Africa. The use of host-plant resistance is an economic, sustainable and environmentally friendly approach to minimizing yield losses caused by FAW under the smallholder farmers' conditions. Development of host plant resistance through breeding is a core component of a comprehensive integrated FAW management strategy, which is needed as a long-term approach to the control of FAW in SSA. Breeding for FAW resistance requires novel genetic resources with resistance genes and suitable agronomic attributes to achieve the maximum possible genetic gains. Over-reliance on commercial hybrids suited to mono-cropping systems has reduced genetic variation in maize and most of the widely cultivated varieties are susceptible to FAW. The narrow genetic diversity and directional selection by breeders has increased crop uniformity over large areas of production, but has also increased the susceptibility of crops such as maize to emerging pests and diseases. Landraces are rich sources of genetic variation and possess novel genes for biotic and abiotic stress tolerance breeding (Acquaah 2009). Horizontal and vertical disease, and pest resistance can be harnessed using landrace maize varieties (Acquaah 2009; Dávila-Flores et al. 2013).

Phenotypic and genomic analyses allow for the selection of desirable genotypes for breeding. Conventional phenotyping entails evaluating genotypes for agronomic performance and selecting the best parents to deliver suitable crosses, and cultivar development (Prasanna et al. 2018). Phenotyping for FAW resistance can be carried out *in situ* under conditions of natural FAW epidemics. This approach involves evaluation of a large number of genotypes in the actual production environments (Mihn 1983). FAW damage levels can be assessed as per Davis et al. (1989). Various molecular marker systems including amplified fragment length polymorphism (AFLP), random amplified polymorphic deoxyribonucleic acid (RAPD), and simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers have been used in genetic analysis of maize (Adu et al. 2019; Barkely et al. 2007; Garcia et al. 2004; Pejic et al. 1998).

Single nucleotide polymorphism markers have gained prominence in genetic diversity studies because of their genomic abundance and flexibility to automation (Inghelandt et al. 2009). Diversity arrays technology sequencing (DArTseq), a next generation sequencing (NGS) method, provides simultaneous, high throughput SNP discovery and genotyping (Baloch et al. 2017). For genetic diversity analysis, Cantelmo et al. (2017) successfully used 23,153

DArTSeq-derived SNP markers to evaluate the genetic diversity of 470 maize inbred lines. DArTSeq markers have also been used to determine the quality of germplasm identity and purity in maize (Chen et al. 2016). There are reports of relative success in identifying genomic regions controlling resistance to several maize diseases, providing vital information for gene identification and genomic selection (Cooper et al. 2019; Gaikpa and Miedaner 2019). Gustafson et al. (2018) successfully evaluated genetic variation in 578 temperate maize inbred lines, identifying genetic variation and genomic regions controlling resistance to sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). There have been reports on the use of SNP markers for genetic variation in resistance to fungal pathogens that cause ear rots, fumonisin contamination and yield loss in maize (Stagnati et al. 2019). Thus far, DArT markers have not been reported as being used to explore genetic variation and selection of maize genotypes for FAW resistance breeding.

Due to the strategic importance of maize for food security, various international research centres and national research programs are geared towards breeding for better maize varieties with improved yield, and multiple resistance to biotic and abiotic stresses. This will enable delivery to farmers of high performing maize cultivars to sustain high productivity and quality (Ndjiondjop et al. 2017). In an attempt to breed for promising maize cultivars with increased yield and FAW resistance, the Zambia maize breeding program at the Zambia Agricultural Research Institute (ZARI) has phenotypically characterized a diverse panel of landraces collected from various growing regions of the country. Preliminary evaluations showed that the test genotypes had variable reactions to FAW infestation and exhibited wide agronomic variation, necessitating well-detailed evaluations to discern the underlying genetic basis using phenotypic and SNP markers. This would determine the genetic diversity and population structure of the population for subsequent use in population improvement and hybrid breeding aiming to create FAW resistant, high yielding and farmer-preferred cultivars. Therefore, the objective of this study was to determine the genetic diversity of 59 maize genotypes of diverse genetic background with variable resistance to fall armyworm, using phenotypic traits and SNP-based DArT markers.

5.2 Materials and methods

5.2.1 Plant materials

The study used a total of 59 maize genotypes consisting of 46 Zambian grown landraces, three obsolete varieties and 10 widely cultivated three-way check hybrids. The landraces were

originally collected from 19 maize growing districts situated in eight provinces with a wide range of agro-ecological conditions. The hybrid varieties were obtained from five seed companies in Zambia. The description of the germplasm used in the study is summarized in Table 5.1.

Table 5.1**.** List of genotypes used in the study and their characteristics

Genotype name	Entry code	Province of collection	District of collection/ releasing authority	Village of collection/ region of cultivation	Local name at village of collection/ category	Reference
ZM 4234	S10	Central	Mumbwa	Ntambo	Gankata	NPGRC[a]
ZM 5021	S23		Kabwe	Chipande	Gankata	
ZM 5043	S49		Serenje	Musonda	Chila	
ZM 4337	S1	Eastern	Lundazi	Zemba	Vingoma	NPGRC
ZM 4336	S19		Lundazi	Malomo	Vingoma	
ZM 4318	S12		Chipata	Nitailileni	Kapesi	
ZM 4329	S3		Chipata	Musukwala	Chimanga	
ZM 4327	S5		Chipata	Chizuma	Mpwera	
ZM 4325	S13		Chipata	Kamusisi	Muumbu	
ZM 4323	S22		Chipata	Kapili	Nyanulanga	
ZM 4316	S18		Chipata	Katokoli	Chimanga	
ZM 4310	S25		Chipata	Mwanaalilenji	Mukangala	
ZM 4308	S35		Chipata	Mwanalilenji	Kafumbu	
ZM 4307	S38		Chipata	Mwanalilenji	Chimanga	
ZM 4312	S32		Chipata	Pwaka	-	
ZM 4324	S39		Chipata	Chatuluka	Muumbu	
ZM 4358	S2		Chadiza	Khamari	Chimanga	
ZM 4321	S21		Katete	Salati	Chimanga	
ZM 7305	S4	Luapula	Mansa	Chembe	-	NPGRC
ZM 7236	S45		Mansa	-	-	
ZM 4333	S50		-	-	-	
ZM 4353	S6	Muchinga	Chama	Chitimbe	Vingoma	NPGRC
ZM 4342	S33		Chama	Taulo	Vingoma	
ZM 4352	S48		Chama	Mundalanga	Vingoma	
ZM 3676	S29		Isoka	Mpandwa	Kanyelenyele	
ZM 7421	S17	Northern	Mbala	-	Unimambwe	NPGRC
ZM 7114	S40	North-western	Solwezi	Lusambu	Yachikaonde	NPGRC
ZM 7147	S41		Solwezi	Maheba camp	-	
ZM 4748	S42		Kabompo	Mumbeji	Pungn	
ZM 4250	S11	Southern	Monze	Chilala	Kampelia	NPGRC
ZM 4255	S14		Monze	Lweendo	-	
ZM 4253	S37		Monze	Chilala	Hickory	
ZM 4252	S30		Monze	Sikanla	Hickory	
ZM 4236	S7		Choma	Elifasi	Silumtuba	
ZM 4264	S9		Choma	Chidakwa	Silutuba	
ZM 4258	S28		Choma	Siachya	Intonga	
ZM 4251	S31		Choma	Simunchembo	Hickory	
ZM 6868	S20		Gwembe	Masawo	-	
ZM 4261	S8		Kalomo	Siasalumba	Yachitonga	
ZM 4261-1	S44		Kalomo	Siasalumba	Yachitonga	
ZM 4237	S36		Namwala	Shanyezhi	-	
ZM 4256	S46		Namwala	Chaambwa	Siluntuba	
ZM 4235	S47		Namwala	Shinchelwe	Hickory king	
ZM 4249	S43		Namwala	Shaloba	Gankata	
ZM 4245	S24		Namwala	Shakopa	Gankata	
ZM 4271	S34		Sinazongwe	Vivawa	Nkaile	
MM 441	S16	Lusaka	ZARI	I & II	Old ZARI varieties	Masole and Gumbo (1994); Howard and Mungoma (1996)
Pool 16	S26		ZARI	I & II		
MM 400	S27		ZARI	I & II		
MM 501	S60		ZARI	I & II		
PHB 30G19	S58	Lusaka	Pioneer	II	Popular hybrids in Zambia	Smale et al. 2013; Mubanga et al. (2018)
DKC 8033	S59		Monsanto	I & II		
ZMS 606	S51		Zamseed	I & II		
DKC 9089	S52		Monsanto	I & II		
SY 5944	S53		MRI-Syngenta	II & III		
DKC 8053	S54		Monsanto	I & II		
ZMS 638	S55		Zamseed	I & II		
DKC 777	S56		Monsanto	I & II		
PAN 7M 83	S57		Pannar	III		

S1-S59 = Sample code for genotype; [a] (NPGRC), National Plant Genetic Resources Centre (http://www.fao.org/pgrfa-gpa-archive/zmb/nfp html); Zambian Agricultural Research Institute (ZARI); MRI-Syngenta- Maize Research Institute-Syngenta seed company; - = Not available; Regions I, II and III denote Agro-ecological regions I, II and III of Zambia, respectively

5.2.2 Phenotyping, data collection and data analysis

Test genotypes were evaluated under field conditions for agronomic traits and FAW reaction to complement the marker data. A field trial was established in Zambia at Mount Makulu Research Station (15°32.87S 28°14.92E, with an altitude of 1225 m above sea level) during the 2017-2018 cropping season following recommended standard practices (Mueller and Sisson 2013). A 6 × 10 alpha lattice design with three replications was used in the evaluation of the genotypes. The seeds were sown in two 3 m rows, with intra- and inter-row spacing of 0.3 m and 0.75 m, respectively. A net plot size of 4.5 m^2 (hereafter referred to as a plot) was used for each genotype.

Days-to-anthesis (DTA) and days-to-silking (DTS) were recorded as the number of days from emergence to the date when 50% of the plants in a plot had produced pollen and silks, respectively. Anthesis-silking-interval (ASI) was calculated as the difference between DTA and DTS. Plant height (PH) expressed in centimetres was measured from 10 representative plants per plot. PH was measured from the soil surface to the top of the tassel of 10 representative plants selected from each plot. Ear position (EP) was measured in centimetres as the length from the ground to the position of the first ear of 10 representative plants in each plot. Ear aspect (EA) was visually assessed and scored on a scale of 1-5, where 1 represented a well formed ear, and 5 was the worst ear in terms of grain-filling, grain type and grain texture. FAW leaf damage (FLD) was recorded as the mean percent leaf damage from 10 representative plants per plot using an FAW damage rating scale. The rating scale was a modification of the Davis et al. (1989) scale, in which leaf damage was assessed on percent basis rather than on a scale of 1 to 9. The present procedure involved careful examination of 10 representative plants per 4.5 m^2 plot for FAW feeding damage. Each of the 10 plants were assigned a class based on the percent of the leaf area damaged by FAW. The number of plants in each class was then multiplied by the median damage percent of that class and the sum of the resultant values was divided by 10 to provide the final percent rating of a genotype per plot. Ear rot (ER) infection was recorded as the number of ears from the 10 sampled plants in each plot that showed symptoms of ear rot disease. FAW cob damage (FCD) was recorded at harvest as the number of ears with more than half of their kernels showing FAW induced damage per 4.5 m^2 plot, expressed as a percentage of the ears of the 10 sampled plants from each plot. Field weight (FW) was recorded as the weight in kilograms of all the ears harvested from the 10 sampled plants in a plot. Grain yield (GY) was calculated per plot as follows;

$$[FW * \left(\frac{10000}{Plot\ Area}\right) * \left(\frac{(100 - GM)}{(100 - 12.5)}\right) * SP]$$

The above data were subjected to analysis of variance after testing for normality and homogeneity of variance using the 18th edition of Genstat (Payne 2015). The data was analysed using a linear mixed model and subjected to the restricted maximum likelihood (REML) procedure in DeltaGen (http://agrubuntu.cloudapp.net/PlantBreedingTool/) to compute the variance components. The landraces were considered as random factor, while the checks (varieties and hybrids) and replicates were treated as fixed factors. Means were separated using Fisher's Unprotected least significant difference at $p \leq 0.05$.

5.2.3 Genotyping

5.2.3.1 DNA Extraction

Total genomic DNA of all the genotypes was extracted at the National Institute for Scientific and Industrial Research (NISIR) biotechnology laboratory in Lusaka, Zambia. The DNA was extracted by the Cetyl-tetramethyl ammonium bromide (CTAB) procedure. Ten average-sized seeds for each genotype were ground to a fine powder using a Geno grinder (MM 200, Retsch, Germany) set at maximum speed for 15 minutes. The powder was mixed with 300 µl sterile deionised water. A uniform 200 mg of grain powder was mixed with 700 µL of CTAB buffer, then incubated for one hour at 65°C and centrifuged for 10 minutes. The supernatant was then transferred into new test tubes, and 500-µl chloroform: iso-amyl alcohol (24:1) was gently mixed into the tubes. After a second round of centrifugation, the DNA was precipitated from the aqueous layer by the addition of salt and 70% ethanol. The upper aqueous phase containing DNA was carefully decanted into a microfuge tube. The resultant DNA pellets were dried in a laminar flow and re-dissolved in 100 µl sterile water. After extraction, the nucleic acid concentration and purity of the DNA was checked using a NanoDrop 2000 spectrophotometer (ND- 2000 V3.5, NanoDrop Technologies, Inc.) following the steps outlined by Desjardins and Conklin (2010).

5.2.3.2 Single nucleotide polymorphism markers

An estimated 20µl of DNA sample of each genotype with concentrations between 50 and 100 ng ul^{-1}, and absorbances ranging from 1.75 to 2.05 were sent to Biosciences Eastern and Central Africa-International Livestock Research Institute (BeCA-ILRI), Kenya for high density and

high throughput genotyping in March 2017. The quality of DNA was checked by incubating the DNA in a digestion buffer for an hour and running 1µl of each sample on 0.8% agarose gel. Afterwards, the genotypes were sequenced using the Diversity Arrays Technology (DArTseq) TM technology, following the procedure described by Akbari et al. (2006).

5.2.3.3 SNP filtering

Genotyping outputs in binary format were converted to HapMap format prior to analysis. The initial 35,701 SNPs from the GBS pipeline were filtered by imputation to remove SNPs with >20% missing data and <5% minor allele frequency (MAF). Missing marker data were imputed using the forest imputation method on the KDCompute sever (https://kdcompute.igss-africa.org/kdcompute/). A total of 27,000 informative SNP markers were used after data imputation. Individual genotypes with >20% missing data were removed.

5.2.3.4 Analysis of genetic diversity parameters

The polymorphic information content (PIC), minor allele frequency (MAF), heterozygosity (Ho) and gene diversity (GD) were calculated using Power-Marker V3.2.5. Analysis of molecular variance (AMOVA) was conducted on the eight populations assembled from eight districts from different sources of collection, using R software version 3.6.1 (R Core Team 2017).

5.2.3.5 Population structure analysis

The population structure of the 59 genotypes was assessed using the admixture model-based clustering method in STRUCTURE version 2.3.4 (Earl and von Holdt 2012). The burn-in period and Markov Chain Monte Carlo (MCMC) iterations were set at 10,000 to derive the population structure based on 27,000 SNP markers distributed across the maize genome. The K-value was set between 1 and 10 to generate the number of subpopulations in the accessions. The best K-value with the highest likelihood for estimating a suitable population size for the dataset was determined using the Evanno procedure (Evanno et al. 2005). Maize genotypes with affiliation probabilities (inferred ancestry) ≥ 80% were assigned to a distinct population, and those ≤ 80% were treated as admixtures, i.e. those genotypes showing a mixed ancestry from identified parents that belong to different gene pools or geographical origins. The genetic dissimilarity matrix was then used to generate the dendrogram based on the Neighbour-Joining (NJ) algorithm. In addition, a principal coordinate analysis (PCoA) was performed to

complement clustering revealed by the dendrogram. The dendrogram was constructed using the KDCompute software (https://kdcompute.igss-africa.org/kdcompute/).

5.3 RESULTS

5.3.1 Phenotypic trait analysis

Analysis of variance revealed highly significant differences (< 0.001) for days to 50 % anthesis and silking, plant height, ear position, ears harvested per plot, ear aspect, ear rot, field weight, grain yield, FAW leaf and cob damage among the 59 genotypes (Table 5.2). Genetic variance were highly significant (≤ 0.001) for plant height, ear position, field weight and grain yield. Anthesis-silking-interval and the two FAW traits showed significant differences (≤0.05). Broad-sense heritability estimates for assessed traits ranged between 0.24 and 0.93 (Appendix 5.1). High heritability (> 0.50) estimates were exhibited by days to 50% anthesis and silking, field weight, grain weight plant height and ear position. Moderate to low (≤ 0.50) heritability estimates were exhibited by FAW cob damage, anthesis-silking-interval, mean FAW leaf damage, ear aspect and ear rot.

Agronomic and FAW damage parameters of the top 15 and bottom five maize genotypes is summarised in Table 5.3, whereas data on the performance of all the genotypes used in the study is presented in Appendix 5.2. Frequency distributions for the measured traits in the study were all symmetrical, with the standard deviation values ranging from to 0.5 to 27.1 (Appendix 5.3). The mean performance of the genotypes shows that days to 50 % anthesis and silking ranged from 53 to 74 days and 55 to 76 days, respectively (Table 5.3). The earliest and latest genotypes were Pool 16 (53 days) and ZM 4316 (74 days). The genotypes DKC 9089 and ZM 4253 had the lowest and highest ear rot values of 7.1 and 57.4%, respectively. The FAW leaf damage (FLD) ranged between 24.5 and 41.7 %. The genotype DKC 8053 had the least symptoms of FAW leaf damage, while ZM 4261-1 was the most susceptible. FAW cob damage ranged from 6.0 to 55.0 % with a mean of 24.3%. Grain yield ranged from 0.9 and 7.3 t ha^{-1}, with PHB 30G19 being the highest yielding genotype.

5.3.1.1 Relationships among assessed phenotypic traits

The highest and positive correlations were observed between days to 50% anthesis and silking. Moderately high and positive correlations were observed between plant height and ear position, plant height and days to 50% anthesis and silking, ear position and days to 50% anthesis and silking.

Table 5.2 Analysis of variance and significance tests for agronomic and FAW damage traits for 59 maize genotypes

Source of variation	df	DTA	DTS	ASI	PH	EP	EA	ER	FW	GY	FLDMean	FCD
Replication (Rep)	2	26.19	31.94	0.35	2987.50	1086.90	2.22	1649.80	15.43	16.98	459.13	472.80
Rep.Block	25	2.13	2.29	0.18	236.1	254.60	1.08	131.30	0.78	0.94	33.60	207.60
Genotype	58	35.07***	41.62***	0.32*	1679.30***	1104.20***	2.57***	365.67***	7.84***	8.97***	40.20***	340.04***
Residual	90	2.01	2.22	0.20	222.40	256.90	0.85	133.90	0.61	0.72	11.08	159.00
LEE	89	2.05	2.25	0.20	226.94	258.60	0.91	134.44	0.64	0.76	13.31	169.18

df - degrees of freedom; *, **, and *** denote significant differences at $p<0.05$, $p<0.01$ and *** $p<0.001$, in that order; LEE - Lattice Effective Error; DTA - days to 50% anthesis; DTS - days to 50% silking; ASI - anthesis silking interval; PH - plant height; EP - ear position; EA- ear aspect; ER - ear rot reaction; FW- field weight; GY- grain yield; FLDMean - mean FAW leaf damage; FCD - FAW cob damage

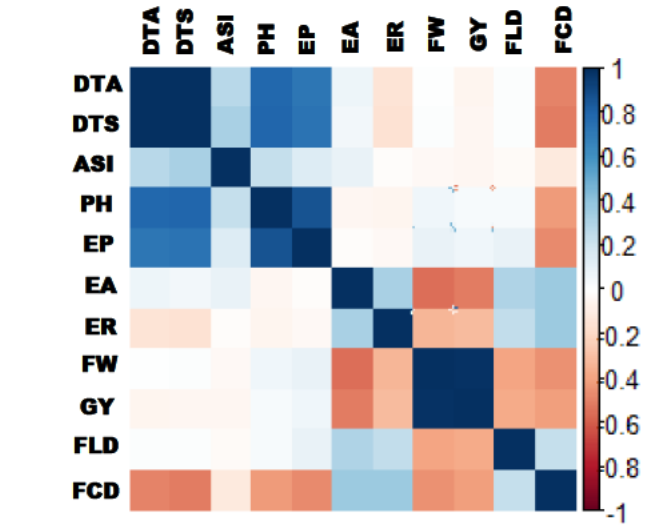
Table 5.3. Mean values for agronomic and FAW damage traits of the top 10 and bottom five maize genotypes based on FAW leaf damage

Genotype	DTA (Days)	DTS (Days)	ASI Days	PH (cm)	EP (cm)	EA (Score)	ER (%)	FW (Kg)	GY (t ha^{-1})	FLDMean (%)	FCD (%)
Top 15 genotypes											
DKC 80-53	62	64	2	176.71	83.33	1.98	18.37	6.08	6.22	24.50	19.97
Pool 16	53	55	2	130 25	61.67	4.00	14.31	0.88	0.96	24.52	25.53
DKC 777	64	66	1	199.41	110.05	2.35	8.70	7.24	7.12	25.87	19.10
PHB 30G19	62	64	2	218 35	125.03	1.30	11.00	7.27	7.32	27.71	6 53
ZM 6868	70	72	2	231.63	106.68	3.36	23.01	3.68	3.79	29.16	10.71
ZM 4358	69	72	3	233.61	111.75	4.00	20.11	2.87	2.94	29.32	22.20
ZM 7305	70	72	2	220 13	106.72	3.28	39.39	2.13	1.94	30.00	11.77
ZMS 606	62	64	2	188 52	86.65	2.30	21.44	5.76	6.30	30.10	30.15
DKC 9089	64	65	1	160 11	84.96	1.61	7.06	6.03	6.55	30.27	23.72
ZM 4748	65	66	2	183 34	90.03	3.62	30.24	2.73	2.85	30.39	28.09
Bottom 5 genotypes											
ZM 4352	68	70	2	221.66	128.30	4.02	29.91	2.06	1.91	38.41	15.14
ZM 4252	66	68	2	236 53	133.35	3.07	31.77	1.72	1.72	39.04	21.97
ZM 4342	63	65	2	168 32	84.93	4.67	21.75	1.45	1.61	40.30	33.81
ZM 4336	66	68	2	206 96	124.97	3.74	43.25	2.60	2.67	40.49	18.95
ZM 4261-1	62	64	2	188 34	98.31	4.32	23.63	2.07	2.15	41.67	33.89
Mean	66	68	2	208 93	111.70	3.65	29.20	3.00	3.04	33.83	24.28
CV (%)	2.15	2 19	22.39	7.14	14.40	25.38	39.42	25.88	27.60	9.80	52.04
LSD (5%)	2.28	2.40	0.73	24.12	25.99	1.498	18.52	1.25	1.36	5.35	20.43
SE	1.41	1.49	0.45	14.94	16.11	0.78	11.48	0.78	0.84	3.32	12.66
SED	1.17	1 23	0.36	12.30	13.13	0.92	9.47	0.66	0.71	2.98	10.62

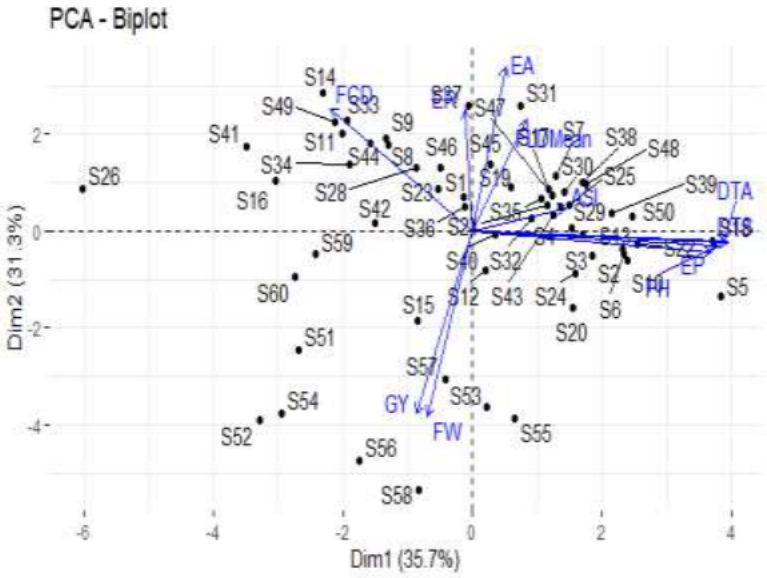
CV- Coefficient of variation; LSD- Least significant difference; SE- Standard error; SED- Standard Error of a difference; DTA-days to 50% anthesis; DTS-days to 50% silking; ASI-anthesis- silking interval; PH- plant height; EP- ear position; EA- ear aspect; ER-ear rot; FW- field weight; GY- grain yield; FLDMean- mean FAW leaf damage; FCD- FAW cob damage

FAW cob damage had a high and negative correlation with days to 50% anthesis and silking, ear position, plant height, field weight and grain yield. Ear aspect also had a high and negative correlation with both field weight and grain yield. FAW leaf damage had a moderately high and negative correlation with both field weight and grain yield. The two FAW parameters, FAW leaf damage and FAW cob damage had a low and positive correlation. Fig. 5.1A.

The relationships among the assessed phenotypic traits depicted by the heat map (Fig. 5.1A) are similar to the patterns presented by the unidirectional vectors in the PC biplot (Fig 5.1B). The contributions of genotypes to a particular trait are shown in the PC biplot (Fig. 5.1B). The genotypes PAN 7M 83 (entry S58) and ZMS 638 (S56) contributed highly towards favourable ear aspect. ZM 4253 (S37) was associated with high ear rot damage. The genotypes DKC 777 (S57), ZMS 638 (S56) and PHB 30G19 (S58) were the greatest contributors to higher grain yield. Genotype ZM 7421 (S17) was associated with high FAW leaf damage, while ZM 4255 (S14) had increased FAW cob damage (Fig. 5.1B).



A



B

Figure 5.1. Correlations among the 11 phenotypic traits assessed in 59 maize genotypes. A- Heat map depicting positive (blue squares) and negative (red) correlations. B- Relationships among the evaluated phenotypic traits and the 59 maize genotypes on a two-dimensional biplot.

DTA- days to 50% anthesis; DTS- days to 50% silking; ASI- anthesis-silking interval; PH- plant height; EP- ear position; EA- ear aspect; ER- ear rot; FW- field weight; GY- grain yield; FLD mean FAW leaf damage; FCD- FAW cob damage

5.3.2 Genetic analysis

5.3.2.1 Population diversity

The observed heterozygosity ranged from 0.28 to 0.47, with a mean of 0.35, while the expected heterozygosity ranged from 0.28 to 0.59. Minor allele frequency and gene diversity varied from 0.01 to 0.5, with means of 0.21 and 0.29, respectively (Table 5.4). The mean polymorphism information content of the SNP markers was 0.23. Most polymorphic markers had a PIC value of 0.38 and only 0.34 % of the markers exhibited a lack of polymorphism. The lowest, highest and mean fixation indices recorded were -0.62, 0.04 and -0.21, respectively. The allele frequency spectrum plot revealed that 35% of all the alleles across the 59 genotypes had frequencies of ≤ 0.1. About 5% of the allelles had the highest frequency of 0.5 (Figure 5.2).

Table 5.4. Genetic diversity statistics for 59 maize genotypes based on 27000 SNPs

	Genetic parameters					
Statistics	Ho	He	MAF	GD	PIC	F
Lower	0.28	0.24	0.01	0.01	0.0	-0.62
Mean	0.35	0.53	0.21	0.29	0.23	-0.21
Upper	0.47	0.85	0.50	0.50	0.38	0.04

Ho- Observed heterozygosity; MAF-minor allele frequency; GD-gene diversity; PIC- polymorphism information content; F- fixation index

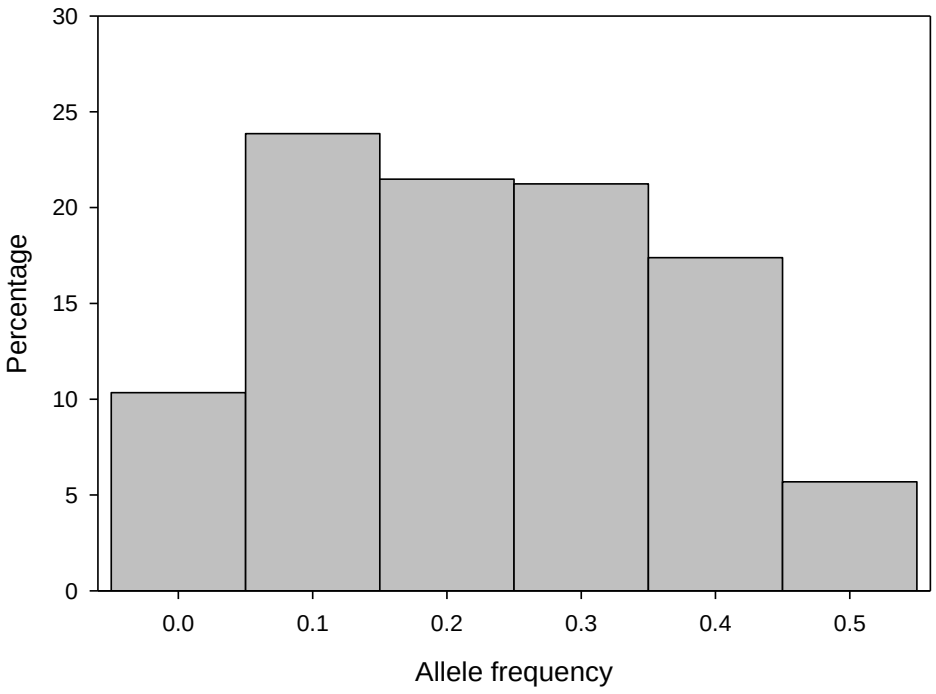


Figure. 5.2. Allele frequency distribution of the 59 maize genotypes assessed with 27 000 SNP markers

5.3.2.2 Population structure

Population structure analysis based on the 27, 000 SNP markers with MAF>0.05 showed that ΔK was highest at K=2, revealing the presence of two main clusters among the accessions (Figure 5.3a and 5.3b). Sub-population I was represented by 79.7% of the test genotypes (Table 5.5). About 75% of the genotypes exhibited membership coefficient values higher than 0.80 in this sub-population, while the remainder could be regarded as admixtures of the smaller sub-populations. Genotypes in Sub-populations I and II had heterozygosity values of 0.11 and 0.40, respectively. Sub-population I had a higher mean fixation index than Sub-population II (Table 5.5).

Table 5.5. Genetic clusters and their member genotypes, proportion of membership, expected heterozygosity and the mean values of Fst observed from structure analysis of 59 maize genotypes

Cluster	Genotypes[a]	Membership %	Expected heterozygosity	Mean fixation index
I	S1, S10, S11, S12, S13, S14, S16, S17, S18, S19, S20, S2, S21, S22, S23, S24, S25, S28, S29, S3, S30, S31, S32, S33, S34, S35, S36, S37, S38, S39, S4, S40, S42, S43, S44, S45, S47, S48, S49, S5, S50, S51, S6, S60, S7, S8, S9	80	0.11	0.65

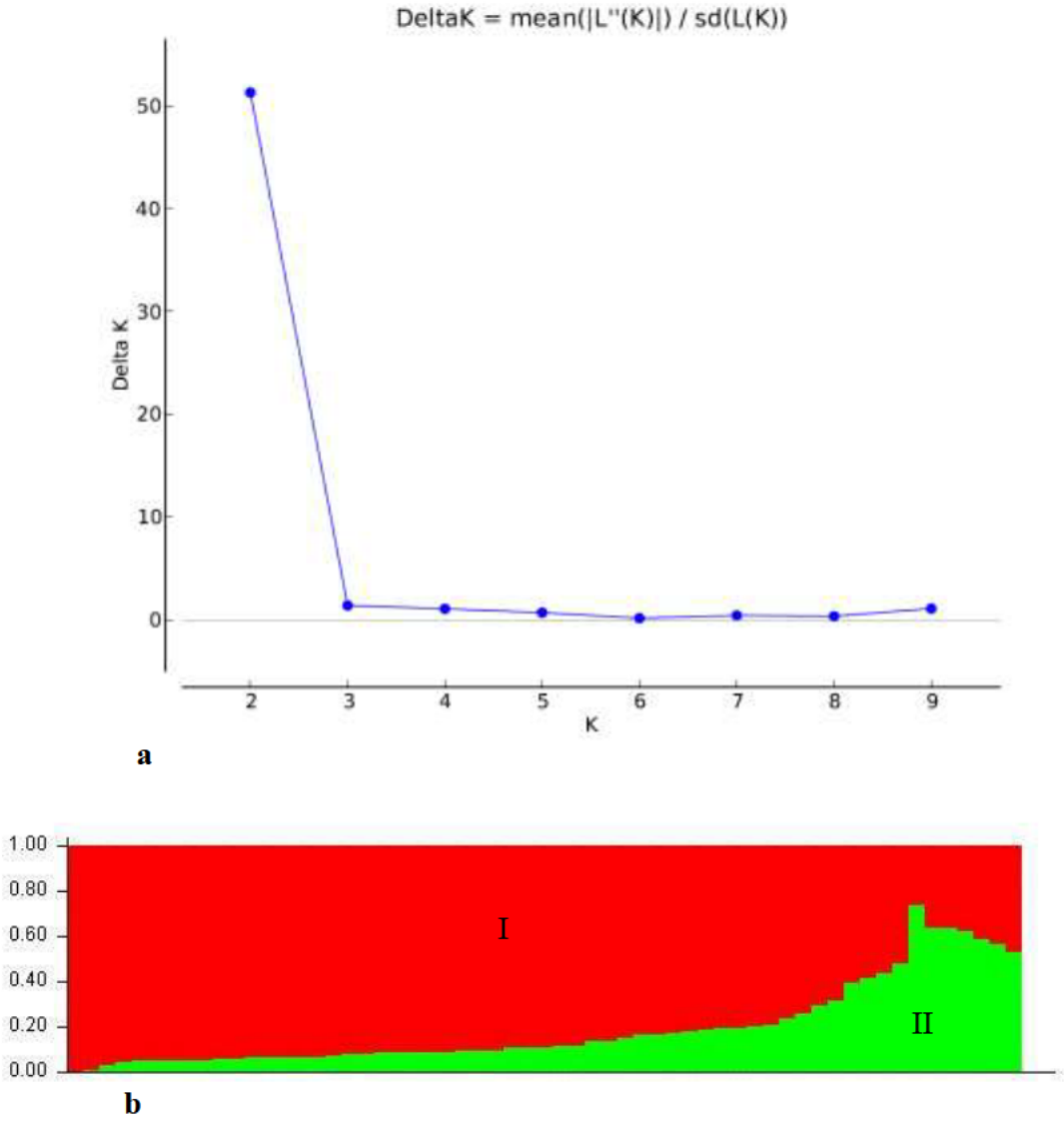


Figure 5.3. Population structure of 59 maize genotypes with two sub-populations as revealed by 27, 000 SNP markers: **a** Delta K estimation based on the Evanno procedure, and **b** Sub-populations I and II for the best delta K value of 2. Genotypes in the upper and lower layers represent sub-populations I and II, respectively

5.3.2.3 Genetic diversity of populations

The neighbour joining dendogram delineated the genotypes into two main clusters (Figure 5.4). Cluster I had 20 genotypes, while Cluster II had 39 genotypes. The two main clusters were further separated into sub-clusters. Genotypes DKC 8033 (S59) and ZM 7421 (S17) were the most distantly related genotypes based on the nighbour joining dendrogram. FAW leaf damage ratings for both DKC 8033 at 32.2% and ZM 7421 (31.4%) were below the grand mean (33.8%) of the test genotypes. The two closely situated genotypes, i.e., ZM 3676 (S29) and Pool 16 (S26) between Clusters I and II had relatively lower ratings for FAW cob damage at 27.6% and

25.5%, respectively. A number of genotypes collected from the same province were closely clustered in the dendrogram. The genotypes ZM 4234 (entry S10) and ZM 5021 (S23); ZM 4327 (S5) and ZM 4325 (S13); and ZM 4329 (S3) and ZM 4316 (S18), which were collected from Chipata district in the Eastern province, were clustered closely (Figure 5.4). The six genotypes (except ZM 5021) were associated with increased plant height, ear position, days to 50% anthesis and silking (Figure 5.1B). Similarly, ZM 4236 (S7), ZM 4253 (S37) and ZM 4235 (S47), which were collected from the Southern province, were clustered closely. ZM 4236 and ZM 4235 were associated with high ratings for ear aspect and FAW leaf damage, while ZM 4253 and ZM4235 were associated with increased ear rot damage (Figure 5.1B). The genotypes ZM 4342 (S33) and ZM 4352 (S48), collected from Muchinga province, were clustered closely and both had FAW leaf damage ratings above the mean of the test genotypes (Appendix 5.2). Within and between populations genetic differences were highly significant ($p = 0.001$), with greater variation being accounted for among genotypes (Table 5.6).

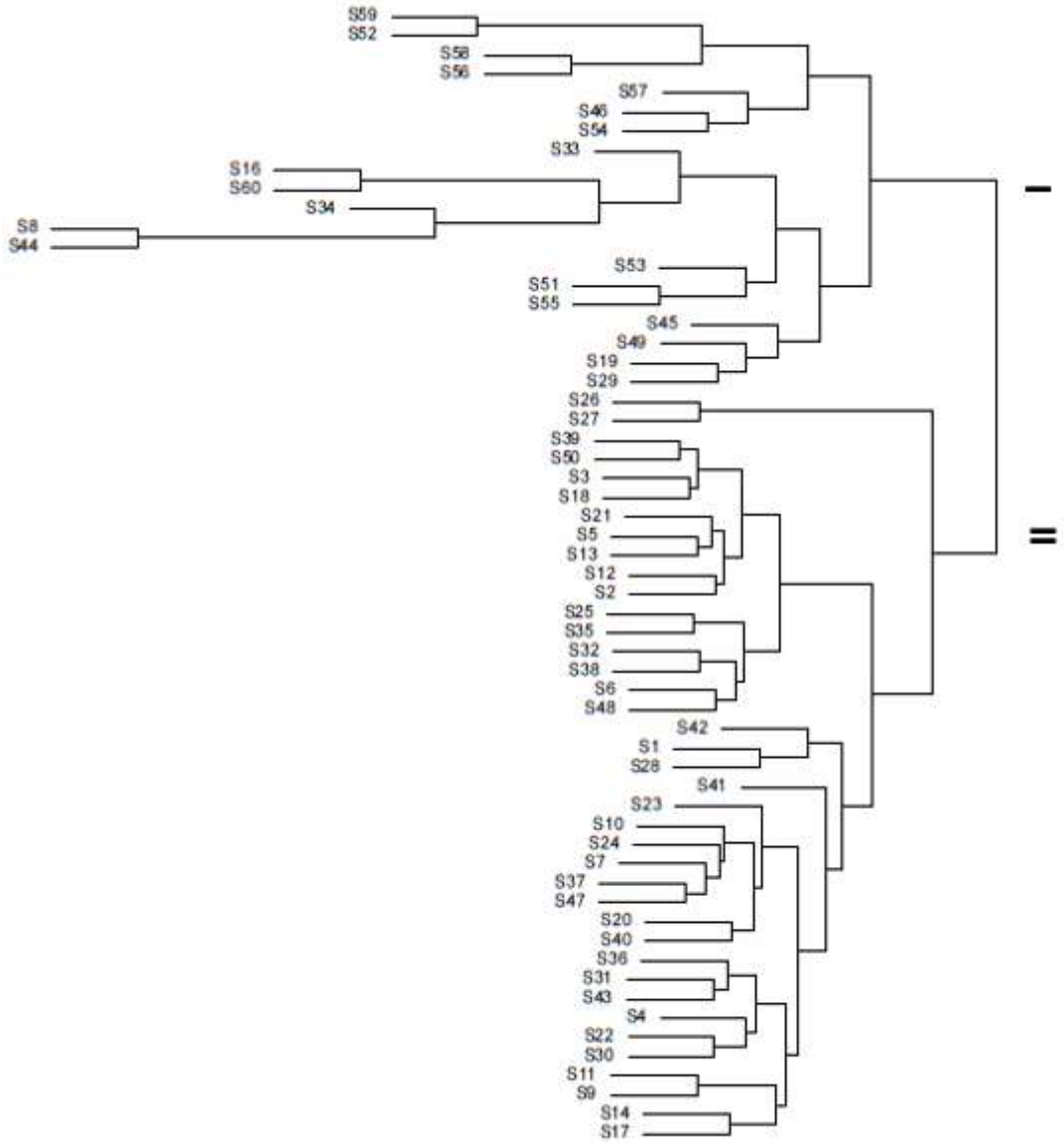


Figure 5.4. Dendrogram showing the genetic relationships among 59 maize genotypes. Note: I and II denote populations I and II based on genotyping with 27,000 SNP markers

S1-S60 represent the genotype entry codes listed in Table 5.1

Table 5.6. Analysis of molecular variance among and within the 59 maize genotypes assessed with 27000 SNP markers

Source of variation	Df	MS	Est. Var.	Proportion of variation (%)
Among populations	7	1193.17	82.21***	11
Within populations	51	643.05	643.00***	89
Total	58		725.17	

Df- Degrees of freedom; MS- mean square; Est.var- Estimated variance; ***- significant at p<0.001

5.3.2.4 Genetic distance

Inbreeding coefficients ranged from -0.50 to -0.17, with a mean of 0.33 representing the population pairs such as G3 and G5, and G3 and G8 (Table 5.7, top diagonal). The pairwise genetic distances among the populations ranged from 0.05 to 0.16, with a mean of 0.08 (Table 5.7, bottom diagonal). Populations G1 and G3, representing genotypes mostly belonging to Cluster I (Figure 5.4), were the most distantly related, while the genetic distance between populations G5 and G6, and G6 and G7 was relatively the shortest. Population G5 consisting of the genotype ZM 4342, was associated with high FAW cob damage revealed by the correlation between traits and genotypes in the PC biplot (Figure 5.1B). Population G6 contained some genotypes with the highest FAW leaf damage and ear rot damage ratings including ZM 4236 (FLD of 35.21%) and ZM 4253 (ER of 57.30%) (Appendix 5.2). The genetic distances between population pairs was below 0.1 for 85.71% of the pairs, suggesting moderate levels of fixation of selected alleles across the populations.

Table 5.7: Pairwise inbreeding coefficients (upper diagonal) and genetic distances (lower diagonal) among eight populations resulting from the 59 maize genotypes based on 27,000 SNPs

	Fis (inbreeding coefficient)							
Populations	G1	G2	G3	G4	G5	G6	G7	G8
G1	-	-0.32	-0.48	-0.37	-0.46	-0.32	-0.46	-0.19
G2	0.07	-	-0.32	-0.30	-0.33	-0.30	-0.33	-0.23
G3	0.16	0.08	-	-0.38	-0.50	-0.32	-0.49	-0.18
G4	0.09	0.06	0.13	-	-0.39	-0.30	-0.38	-0.17
G5	0.10	0.07	0.14	0.08	-	-0.33	-0.48	-0.20
G6	0.05	0.05	0.06	0.05	0.05	-	-0.33	-0.24
G7	0.08	0.07	0.09	0.07	0.07	0.05	-	-0.20
G8	0.10	0.07	0.14	0.08	0.08	0.05	0.07	-
	Fst (genetic distance)							

Note: G1 to G7 represent popoulations sampled from the following provinces in Zambia: G1- Central; G2- Eastern; G3- Luapula ; G4- Lusaka; G5- Muchinga; G6- Southern; G7- North-Western, while G-8 are commerial hybrids.

The genetic parameters for the eight geographically based population groupings are shown in Table 5.8. The gene diversity among the eight test populations ranged from 0.22 to 0.28, with the lowest and highest values belonging to G3 and G6. The PIC values ranged from 0.17 to 0.22 with three populations, G2, G6 and G8, having the highest PIC value of 0.22. The MAF and Ho ranged from 0.18 to 0.21 and 0.3 to 0.39, respectively.

Table 5.8: Genetic diversity parameters of 59 maize genotypes generated using 27,000 SNP markers

Population	Genetic parameters				
	GD	PIC	MAF	Ho	F
G1	0.26	0.21	0.20	0.37	-0.44
G2	0.27	0.22	0.20	0.35	-0.31
G3	0.22	0.17	0.18	0.34	-0.51
G4	0.27	0.21	0.20	0.35	-0.31
G5	0.26	0.21	0.21	0.39	-0.49
G6	0.28	0.22	0.20	0.36	-0.28
G7	0.27	0.21	0.21	0.39	-0.45
G8	0.27	0.22	0.19	0.30	-0.10

Note: G1 to G7 represent popoulations sampled from the following provinces in Zambia: G1- Central; G2- Eastern; G3- Luapula ; G4- Lusaka; G5- Muchinga; G6- Southern; G7- North-Western, while G-8 are commerial hybrids. Ho- Observed heterzygoosity; MAF-minor allele frequency; GD-gene diversity; PIC- polymorphism information content; F- fixation index

5.4 Discussion

The present study found significant differences for key agro-morphological traits and FAW resistance when assessing 59 maize genotypes (Tables 5.1 and 5.2). This suggests that the assembled germplasm was genetically diverse and would be useful for maize improvement programs in Zambia or similar agro-ecologies. The range of genetic variability for various traits is genotype dependent. Quantitatively inherited traits such as grain yield, plant height, anthesis and silking interavls have polygenic inheritance and exhibit continuous and wide variation (Mackay 2009), which can facilitate selection between genotypes in a given population. Conversely, traits with qualitative inheritance are governed by fewer genes whose effects manifest in a narrow range of variation (Geleta and Lubuschagne 2005). Information on the magnitude of relationships between measured traits enables simultaneous or indirect selection for trait pairs with either positive or negative correlations. In the present study, traits including plant height, ear position, days to 50% anthesis had highly positive correlations, suggesting that direct selection of one trait would concurrently improve the other traits. Conversely, field weight and grain yield had highly negative correlations with FAW cob damage suggesting indirect selection among these traits (Ziyomo and Bernado 2013). Pest and disease resistance governed by major genes show discreet phenotypic classes of resistant and susceptible individuals, whereas quantitative resistance manifests as a continuous spectrum of individuals with varied genetic backgrounds (Cornwin and Kliebenstein 2013). Therefore, the variable FAW reaction types found in the present study harness selection using phenotypic and molecular markers.

The phenotypic variation present in the test germplasm was confirmed through genetic diversity assessement using the analysis of molecular variance (Table 5.6). The observed mean heterozygosity of 0.35, although lower than the expected heterozygosity of 0.53, was favorable for developing open-pollinated breeding populations. A population with moderate heterozygosity is one in which most of the genetic loci are not fixed in a homozygous (Ho=0) or heterozygous (Ho=1) state (Elston 2005). In cross-pollinated species including maize, low heterozygosity may lead to the expression of deleterious recessive alleles and associated susceptibility to biotic or abiotic stresses (Radosavljević et al., 2015).

In this study a mean PIC value of 0.23 was obtained, showing that the SNPs were able to discriminate between the genotypes adequately. The PIC value signifies the usefulness of the test markers, taking into account the population heterozygosity, major allele frequency and gene diversity (Elston 2005). SNPs provide lower PIC values than SSRs (Chao et al. 2009). The mean PIC value in this study was similar to those obtained by Wu et al (2014) and Zhang et al. (2016) who reported a mean PIC value of 0.29 when assessing similar populations of Chinese maize inbred lines in different localities.

The mean gene diversity in this study (0.29) was comparable to the ranges of 0.23-0.32 reported by Inghelandt et al. (2010) when using SNP markers. Wu et al (2014) reported a gene diversity value of 0.23 in a panel of Chinese maize inbred lines. Considering the theoretical gene diversity for bi-allelic markers of 0.5, the gene diversity obtained in this study was higher. This suggests that the genes present in the test populations segregated signficantly. Gene diversity is a reflection of a population's genetic constiutution and adaptability to variable environments (Markert et al., 2010). Populations sampled from the Southern province (G6) had the highest gene diversity of 0.28, which is likely to be more useful in breeding for resistance to FAW damage.

The structure analysis clustered the maize genotypes into two distinct populations, based on their genetic composition, irrespective of their geographical sources of collection. Reports of stratification and clustering of genotypes irrespective of geographical sources are common in genetic studies (Shrestha 2016; Aci et al. 2018). The clustering pattern in this study was partially consistent with differences in the geographical sources of the genotypes. This is shown by the number of genotypes from common geographical locations, particularly from the Eastern, Lusaka, Southern and Muchinga provinces, where geneotypes were clustered closley on the dendrogram (Figure 5.4). In addition to being from common geographical locations, some of the genotypes in the same cluster also shared similar phenotypic characteristics. For

instance PHB 30G19 and DKC 777 clustered close to each other and were also identifed among the highest yielding genotypes in the study. Population stratification based on the underlying genetic constitution is more important and useful for breeding purposes because genetic components are heritable but not geographical delineation. The moderate to high heritability estimates of most of the key agronomic and FAW traits obtained in this study confirm that the results would be repeatable, which is ideal for FAW resistance breeding. The development of hybrids with superior yield potential, client-preferred characteristics and improved FAW resistance could be achieved by crossing suitable parental lines between the genetic clusters rather than across sources of collection. In addition to the similarities in geographic origin, it is also possible to cluster genotypes based on their similarities in some key phenotypic traits, irrespective of their sources (Adu et al. 2019). For instance, Cluster I forms a favourable pool of candidate genotypes. This may allow for simultaneously developing breeding populations with a background of FAW resistance/tolerance and high yield potential due to earliness, a narrow range of ASI, low FCD ratings and high yield expression.

Significant variation within a population was revealed by the analysis of molecular variance (Table 5.6), which implied that there is a possibility of effective selection from a mini-core collection of the test population. Within-population selection enables identification of genotypes adapted to specific climatic conditions or management practices. Liu et al. (2003) asserted that within and between population variation afforded breeders more opportunities to achieve higher genetic gains during crop improvement.

The genetic diversity indices obtained in this study (Table 5.7) suggest that adequate levels of genetic variation exists in the assessed maize collection consistent with the predominantly allogamous mating system of maize (Souza 2011). Among the calculated indices, inbreeding coefficients (Fst) and genetic distances (Fis) (Table 5.7) provided further insight into the nature of the tested maize germplasm. Inbreeding coefficient represents the proportion of genes that were common among individual genotypes within the eight populations, while genetic distance is associated with the phylogenetic relatedness of genotypes. Population Fst values averaged 0.079 (Table 5.7), which is regarded as moderate differentiation according to Wright (1978), Hartl and Clark (1997) and Balloux and Moulin (2002). Moderate differentiation could be attributed to sharing of genetic material among farmers from different locations, given that the germplasm was largely made up of landraces that have been cultivated for many years in and around Zambia. Although landraces are highly heterozygous, their broad adaptation may have fixed many of the genetic loci, resulting in moderate gene differentiation. Romay et al. (2013)

reported lower differentiation among the Corn Belt germplasm with a pairwise mean Fst of 0.04. Conversly, Schaefer and Bernado (2013) reported a mean Fst of 0.17 among maize inbred lines from a diverse collection, implying higher differentiation and less kinship.

The populations that were most genetically distant (G1 and G3) (Table 5.8) were obtained from geographically distant sources (Central and Luapula provinces), implying that there was limited gene flow between the geographically separated germplasm. This was also consistent with the least genetically distant populations (G5 and G6; G6 and G7) (Table 5.8) being obtained from geographically closer regions (Lusaka and Southern; Southern and North-western regions, in that order). In addition to the proximity of geographical sources, climatic conditions and farming practices under which the landraces developed may have impacted their adaptation, resulting in genetic differentiation.

5.5 Conclusion

The assessment of maize landraces using key phenotypic traits and SNP-based DArT markers revealed significant genetic variation valuable for maize improvement. The mean PIC value was 0.23 and heterozygosity was 0.35. The test genotypes were allocated in similar clusters based on the following phenotypic attributes: days to 50% flowering, anthesis-silking-interval, yield expression and level of FAW-related damage. The genotypes were stratified into two sub-populations. About 75% of the test population exhibited a membership coefficient of 0.80 to the largest sub-population. The genetic grouping is key to developing maize cultivars with favourable gene combinations to exploit heterosis for yield and farmer-preferred traits. Overall, the present study selected genetically distinct and unique genotypes such as Pool 16, ZM 4236 and ZM 7114 for their favourable agro-morphological traits and FAW resistance for ongoing breeding of maize varieties suitable for Zambia and similar agro-ecologies.

5.6 References

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5.7 Appendices

Appendix 5.7.1. Variance components for agronomic and fall armyworm (FAW) parameters

Source of variation	DTF	DTA	ASI	PH	EP	EA	ER	FW	GY	FLDMean	FCD
σ^2_g	0.35	0.38	0.04*	0.04***	214.28***	0.20	0.79	0.96***	1.05***	2.64*	1.86*
σ^2_{gr}	8.95	9.59	0.12	0.0203	107.62	0.62	6.94	0.27	0.33	13.47	4.17
σ^2_e	1.81	1.40	0.09	0.00	116.67	0.46	0.76	0.30	0.33	0.06	3.03
Fixed terms						F value					
Check	20.23	20.25	13.28	373.26	31.81	10.80	71.57	53.85	51.82	1041.39	12.94
Replicates	11.36	12.83	1.26	11.33	4.71	2.29	2.09	25.42	23.55	7.71	19.50
Check(Replicates)	0.67	0.83	1.25	6.86	3.13	1.47	7.36	2.93	3.22	541.33	2.36
Heritability	0.93	0.93	0.38	0.83	0.74	0.36	0.24	0.84	0.83	0.37	0.44

σ^2_g - genotype variance; σ^2_{gr} ; genotype by replication variance; σ^2_e - environmental variance; * and *** denote significant differences at $p<0.05$ and $p<0.001$, in that order; DTA - days to 50% anthesis; DTS - days to 50% silking; ASI – anthesis-silking-interval; PH - plant height; EP - ear position; EA- ear aspect; ER - ear rot reaction; FW- field weight; GY- grain yield; FLDMean - mean FAW leaf damage; FCD - FAW cob damage

Appendix 5.7.2. Mean values for agronomic and fall armyworm (FAW) damage traits of the 59 maize genotypes used in the study

Genotype	Sample code	DTA (Days)	DTS (Days)	ASI Days	PH (cm)	EP (cm)	EA (Score)	ER (%)	FW (Kg)	GY (t ha^{-1})	FLDMean (%)	FCD (%)
DKC 8053	S54	62	64	2	176.71	83.33	1.98	18.37	6.08	6.22	24.50	19.97
Pool 16	S26	53	55	2	130.25	61.67	4.00	14.31	0.88	0.96	24.52	25.53
DKC 777	S56	64	66	1	199.41	110.05	2.35	8.70	7.24	7.12	25.87	19.10
PHB 30G19	S58	62	64	2	218.35	125.03	1.30	11.00	7.27	7.32	27.71	6.53
ZM 6868	S20	70	72	2	231.63	106.68	3.36	23.01	3.68	3.79	29.16	10.71
ZM 4358	S2	69	72	3	233.61	111.75	4.00	20.11	2.87	2.94	29.32	22.20
ZM 7305	S4	70	72	2	220.13	106.72	3.28	39.39	2.13	1.94	30.00	11.77
ZMS 606	S51	62	64	2	188.52	86.65	2.30	21.44	5.76	6.30	30.10	30.15
DKC 9089	S52	64	65	1	160.11	84.96	1.61	7.06	6.03	6.55	30.27	23.72
ZM 4748	S42	65	66	2	183.34	90.03	3.62	30.24	2.73	2.85	30.39	28.09
ZM 5021	S23	64	66	2	207.01	103.32	4.26	43.94	2.11	2.17	30.53	16.97
ZM 4249	S43	67	69	3	220.07	125.05	3.65	41.00	2.73	2.57	31.16	22.73
ZM 4318	S12	66	67	1	226.84	135.00	3.30	25.08	3.16	3.19	31.35	25.88
ZM 7421	S17	68	70	3	221.83	111.62	4.28	21.65	2.04	1.97	31.42	31.98
ZM 7114	S40	65	67	2	216.81	121.67	2.60	39.03	2.24	2.32	31.53	16.59
ZM 4253	S37	66	68	2	205.17	111.64	4.61	57.40	1.17	0.93	32.12	32.45
DKC 8033	S59	62	64	2	171.40	78.79	4.56	24.99	3.79	4.02	32.18	18.56
MM 501	S60	62	64	2	180.14	84.97	2.63	49.53	5.00	5.55	32.35	30.19
ZM 4337	S1	66	68	2	213.33	108.33	3.36	33.86	2.47	2.16	32.39	32.64
ZM 4310	S25	69	71	2	213.52	128.33	4.61	23.28	2.04	2.11	32.43	21.04
MM 441	S16	60	62	2	183.51	86.62	4.32	41.89	3.48	3.66	32.81	40.82
SY 5944	S53	66	69	3	208.31	113.31	1.66	19.01	6.59	7.09	33.00	18.49
ZM 4324	S39	70	72	2	227.96	130.04	5.03	17.31	2.38	2.14	33.18	19.60
ZM 4325	S13	72	74	2	221.80	121.66	3.67	19.61	2.85	2.84	33.22	19.61
ZM 4235	S47	67	69	2	221.63	130.01	4.69	37.29	2.67	2.69	33.27	19.00
ZM 4321	S21	67	69	2	230.09	116.66	3.59	27.32	1.84	1.90	33.28	17.84
ZM 4323	S22	69	72	3	223.27	136.63	3.58	24.92	2.77	2.73	33.54	15.33
ZM 4327	S5	74	76	3	233.21	129.98	3.60	13.45	3.48	3.14	33.68	8.97

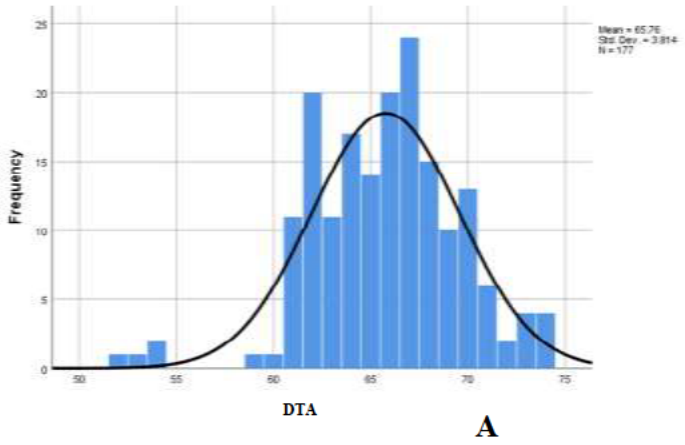
Appendix 5.7.2 Continued

ZM 4329	S3	70	72	2	213.54	116.65	3.97	26.83	2.61	2.50	33.94	11.14
ZMS 638	S55	65	67	2	224.93	138.39	2.45	20.02	6.36	6.74	34.04	6.05
ZM 4307	S38	68	70	2	238.44	139.98	4.56	28.27	2.24	2.24	34.09	33.31
MM 502	S15	63	65	2	206.54	105.02	3.35	20.67	4.66	5.09	34.12	12.64
ZM 4308	S35	68	70	2	224.74	121.65	3.35	40.28	2.07	1.96	34.31	25.06
ZM 4234	S10	69	71	2	251.34	146.72	2.12	41.00	2.80	2.67	34.44	24.21
ZM 7147	S41	62	64	2	155.05	75.04	2.98	21.37	1.38	1.58	34.67	55.02
ZM 4255	S14	61	63	2	193.61	98.30	4.97	48.46	2.09	2.11	34.74	49.67
ZM 4264	S9	62	64	2	209.97	103.31	3.99	39.59	1.83	1.71	34.84	38.80
ZM 4236	S7	67	69	2	234.72	126.69	5.03	43.46	2.75	2.88	35.35	25.01
ZM 4250	S11	63	65	2	185.27	88.31	4.05	38.20	2.07	2.09	35.50	41.96
ZM 4261	S8	65	67	2	193.75	93.32	4.30	25.77	1.79	1.77	35.57	37.50
PAN 7M-83	S57	66	67	2	211.96	109.98	2.37	23.41	6.47	6.61	35.62	19.25
ZM 4237	S36	64	66	2	218.20	113.41	3.33	38.46	2.57	2.53	35.88	20.32
ZM 4245	S24	68	70	2	231.36	128.36	3.11	30.70	3.16	3.12	36.08	13.30
ZM 4256	S46	64	66	2	208.55	113.30	4.69	28.59	2.22	2.25	36.08	33.53
ZM 4312	S32	66	69	2	224.86	126.68	3.40	25.61	2.48	2.47	36.34	26.57
ZM 4251	S31	68	69	2	216.43	118.32	4.96	42.45	1.27	1.30	36.53	38.32
ZM 3676	S29	68	70	2	231.16	120.02	3.69	20.54	2.05	2.00	36.55	27.59
ZM 4271	S34	62	64	2	176.72	89.96	4.05	43.82	2.29	2.30	36.79	22.24
ZM 4316	S18	74	76	2	232.71	140.00	3.64	17.20	2.23	2.14	36.83	13.78
ZM 4258	S28	64	66	2	211.46	101.70	4.13	33.99	2.35	2.43	37.09	37.35
ZM 4333	S50	70	72	2	224.78	140.04	4.35	23.46	2.49	2.32	37.51	16.08
ZM 5043	S49	62	64	2	183.29	95.02	4.68	42.42	2.31	2.33	37.68	40.67
ZM 7236	S45	67	69	2	203.36	101.67	4.34	29.66	1.83	1.87	37.86	22.97
ZM 4353	S6	70	72	2	230.10	128.25	4.03	24.06	3.09	3.31	38.16	10.20
ZM 4352	S48	68	70	2	221.66	128.30	4.02	29.91	2.06	1.91	38.41	15.14
ZM 4252	S30	66	68	2	236.53	133.35	3.07	31.77	1.72	1.72	39.04	21.97
ZM 4342	S33	63	65	2	168.32	84.93	4.67	21.75	1.45	1.61	40.30	33.81

Appendix 5.7.2 Continued

ZM 4336	S19	66	68	2	206.96	124.97	3.74	43.25	2.60	2.67	40.49	18.95
ZM 4261-1	S44	62	64	2	188.34	98.31	4.32	23.63	2.07	2.15	41.67	33.89
Mean		66	68	2	208.93	111.70	3.65	29.20	3.00	3.04	33.83	24.28
CV (%)		2.15	2.19	22.39	7.14	14.40	25.38	39.42	25.88	27.60	9.80	52.04
LSD (5%)		2.28	2.40	0.73	24.12	25.99	1.498	18.52	1.25	1.36	5.35	20.43
SE		1.41	1.49	0.45	14.94	16.11	0.78	11.48	0.78	0.84	3.32	12.66
SED		1.17	1.23	0.36	12.30	13.13	0.92	9.47	0.66	0.71	2.98	10.62

CV- Coefficient of variation; LSD- Least significant difference; SE- Standard error; SED- Standard Error of the mean difference; DTA-days to 50% anthesis; DTS-days to 50% silking; ASI-anthesis-silking-interval; PH- plant height; EP- ear position; EA- ear aspect; ER-ear rot; FW- field weight; GY- grain yield; FLDMean- mean FAW leaf damage; FCD- FAW cob damage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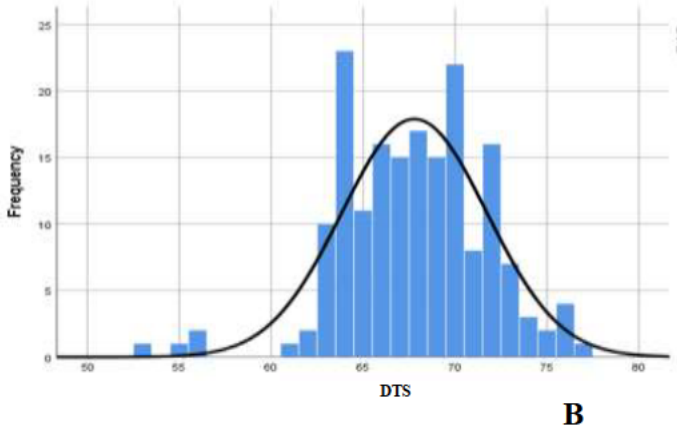
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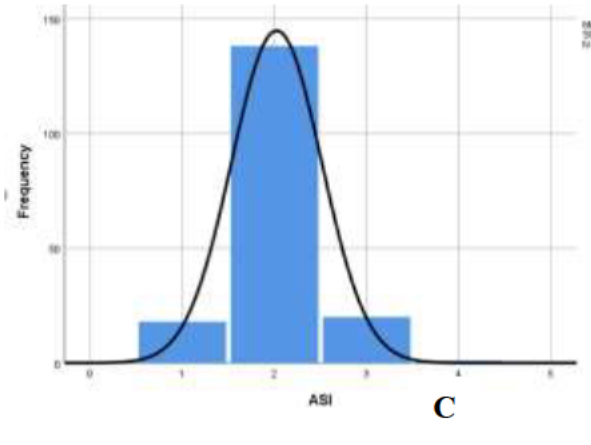
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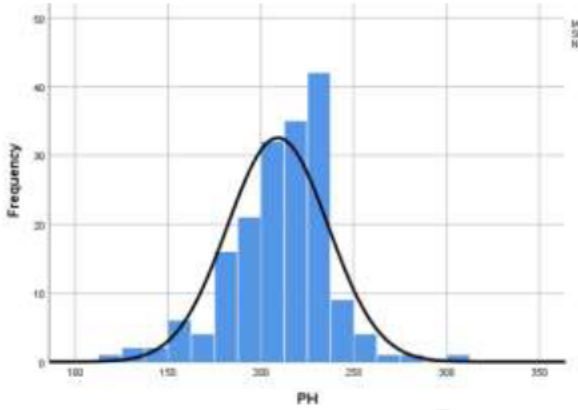
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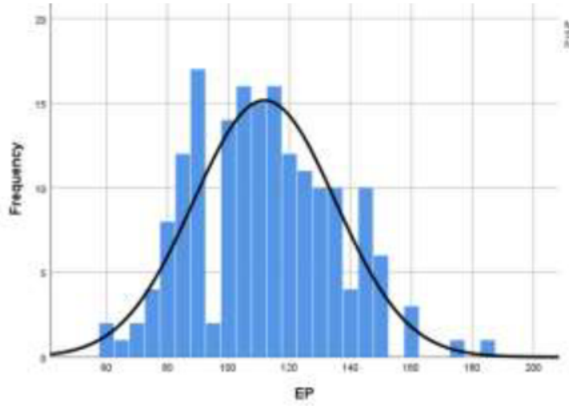
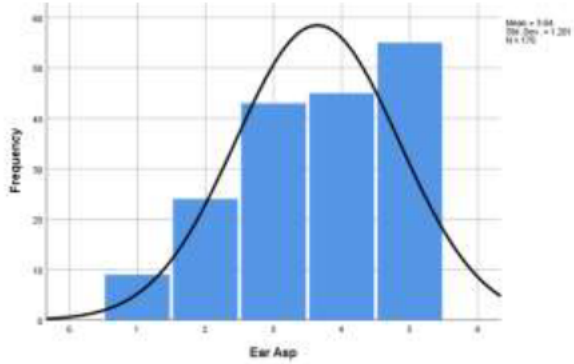
PH

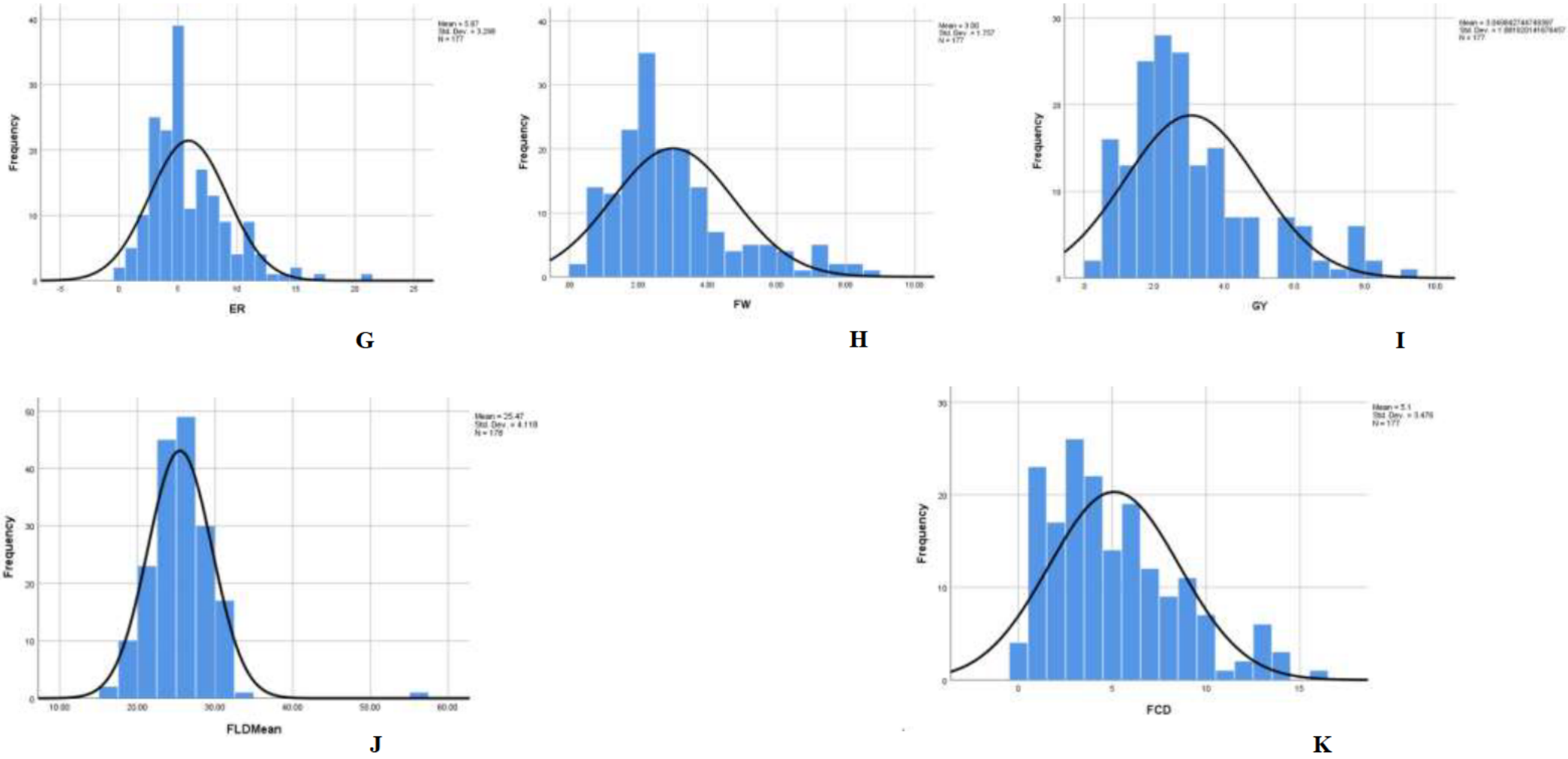
D


Frequency
EP

E


Frequency
Ear Asp

F



Appendix 5.7.3. Frequency distribution of 59 maize genotypes for (A) days to 50% days to anthesis (DTA), (B) 50% days to silking (DTS), (C) anthesis-silking interval (ASI), (D) plant height, (E) ear position (EP), (F) ear aspect (EA) , (G) ear rot (ER), (H) field weight, (I) grain yield (GY), (J) mean FAW leaf damage (FLD Mean), (K) FAW cob damage (FCD)

CHAPTER 6. COMBINING ABILITY OF MAIZE GENOTYPES FOR FALL ARMYWORM (*Spodoptera frugiperda* J.E. Smith) RESISTANCE, YIELD AND YIELD-RELATED TRAITS

Abstract

This study aimed to investigate the combining ability effects and inheritance of fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith) resistance and agronomic traits in maize genotypes for breeding. A line × tester mating design was used and 60 experimental hybrids were generated and field-evaluated, in three FAW hotspot locations in Zambia. Both the general and specific combining ability effects were significant ($p<0.05$) for the assessed traits. Non-additive genetic effects were more important for the inheritance of grain yield and FAW-inflicted leaf and cob damage, suggesting that heterosis breeding would be the best strategy for improving these traits in this population. The narrow sense heritability (h^2) estimates for agronomic and FAW-related traits ranged from 0.14 to 0.47 and 0.37 to 0.49, respectively. The experimental hybrids CML346/EBL16469, ZM4236/CML545-B, CML346/CZL1310c, CML334/EBL173782, CML545-B/EBL169550 were among those selected with favourable specific combining ability estimates for grain yield, days-to-50% anthesis, days-to-50% silking, FAW leaf and cob damage resistance, respectively. The selected experimental hybrids are recommended for further evaluation and breeding in Zambia or similar agro-ecologies.

Keywords: combining ability effects, fall armyworm, heritability, heterosis, line × tester mating design, maize

6.1 Introduction

Maize (*Zea mays* L., 2n = 2x = 20) is the leading cereal crop in sub-Saharan Africa. However, its production is constrained by fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith). The pest causes extensive damage to maize plants resulting in yield losses reaching up to 53% depending on genotype susceptibility, severity of the infestation and the crop stage (Prasanna et al., 2018). An integrated FAW management strategy incorporating host plant resistance is regarded as the most sustainable and cost-effective approach to control the FAW (FAO 2018). Host plant resistance is an essential component, which relies on the inherent defence mechanism of the plant. However, maize varieties that are both FAW-resistant and high-yielding are not available in sub-Saharan Africa, limiting the use of this component in integrated FAW management (Prasanna et al., 2018). There is an urgent need to develop FAW-resistant maize cultivars for Africa (FAO 2018; Kasoma et al., 2020a). Successful development of FAW-resistant maize cultivars requires screening of a large panel of genetically diverse genotypes to identify sources of resistance genes.

Combining ability analysis among promising lines is a prerequisite to identify superior genetic material and deduce gene action (Makumbi et al., 2011; Fasahat et al., 2016). Combining ability effects are divided into the general combining ability (GCA) effect of parents and the specific combining ability (SCA) effect of hybrids (Sprague and Tatum, 1942). The GCA and SCA effects are associated with additive and non-additive gene action, respectively (Falconer, 1996). Traits whose inheritance is conditioned by additive and non-additive gene actions are improved through recurrent selection and heterosis breeding, respectively (Dudley and Moll, 1969; Hallauer, 1988; Bernado, 2020).

Among the common mating designs, the line × tester is a design of choice when assessing the combining ability effects of known testers possessing desirable economic traits (e.g. FAW resistance) and less characterized candidate maize lines. The design maximizes the evaluation of genotypes with varying degrees of partial resistance observed through prior screening (Fasahat et al., 2016). The line × tester design has been used widely to deduce combining ability effects and to estimate genetic variance components for yield, agronomic traits and insect resistance in maize (André et al., 2003; Elmyhun, 2013; Izhar and Chakraborty, 2013; Rahman et al., 2013; Kamara et al., 2014; Rovaris et al., 2014; Ruswandi et al., 2015; Mutimaamba et al., 2019).

Diverse maize genotypes from the International Maize and Wheat Improvement Center (CIMMYT) and from local collections were previously screened for FAW resistance and

agronomic performance. This allowed for the selection of some 25 promising genotypes from the CIMMYT collection and five open pollinated varieties (OPVs) from Zambia (Kasoma et al., 2020b; 2020c). Therefore, the objective of this study was to investigate the combining ability effects and trait inheritance among the selected maize genotypes and to select desirable parents and single-cross hybrids with partial FAW resistance and suitable agronomic traits for effective FAW resistance pre-breeding and breeding programs in Zambia and related agro-ecologies.

6.2 Materials and methods

6.2.1 Plant materials and generation of the hybrids

The study used 17 parental genotypes consisting of 12 lines and five testers selected for FAW resistance and favourable agronomic traits (Masole and Gumbo, 1994; CIMMYT 2019; Kasoma et al., 2020b). Two single cross commercial hybrids, namely MM501 and MM502, were used as comparative control. The checks are moderately resistant to FAW, MSV and leaf blight (Table 6.1). A line × tester mating scheme was used to generate 60 experimental hybrids. The crosses were made in 2018 in Zambia at the Golden Valley Research Trust (GART) farm (14.9659° S, 28.1019° E). Testers were staggered planted at one-week interval to synchronize pollen production and silk formation of the lines for crosses. At the reproductive growth stage, the tassels of the testers were bagged using brown water-proof bags. Similarly, the emerging ear shoots of the lines earmarked for pollination were bagged using clear polyethylene shoot bags prior to silk emergence. Hand pollination was conducted a day after tassel and ear isolation, at between 7:00 and 10:00 am. Recommended agronomic practices including weeding, irrigation and pest management were followed up to crop maturity (Mueller and Sisson, 2013).

Table 6.1. Descriptions of the maize genotypes used in the study

Name or designation	Pedigree/source of germplasm	Source	Parent status	GY t ha^{-1}	FAW reaction and other attributes	Reference
VL05120	CML176-B-B	CIMMYT	Line	-	FAW reaction unknown	CIMMYT (2019)
CML548-B	CML488-B	CIMMYT	Line	3.30	Partial/moderate resistant to FAW	Kasoma et al. (2020b)
EBL169550	([SYN-USAB2/SYN-ELIB2]-12-1-1-1-B*4-B-B-B*/OFP105)-4-2-2-2-2-B-B-B-B	CIMMYT	Line	1.56	FAW reaction unknown	Kasoma et al. (2020b)
TL1512847	(Syn01E2-64-2-B-2-B/CIMCALI8843/S9243-BB-#-B-5-1-BB-4-3-4-B)-B-14-1-2-2-B-B-B	CIMMYT	Line	1.12	FAW reaction unknown	Kasoma et al. (2020b)
EBL173809	[[CML506/[CML205/CML176]-B-2-1-1-B]F2-1/[CML144/[CML144/CL395]F2-8sx]-1-2-3-B*4]-24-B-2-BBB-3-B-B-B-B-B-B	CIMMYT	Line	-	FAW reaction unknown	CIMMYT (2019)
EBL1611480	(CML395IR/([NIP25-20-1-1-B-1-B*4/CML511]-4/CML444IR)-B)F2-B-5-2-1-1-B-B-B	CIMMYT	Line	-	FAW reaction unknown	CIMMYT (2019)
EBL1611469	([CML202/CML144]F2-1-1-3-B-1-B*6/CML511//CML511)-7-3-2-2-2-B-B-B-B	CIMMYT	Line	0.37	FAW reaction unknown	Kasoma et al. (2020b)
TL13905	(CML395IR/([NIP25-175-2-1-BBB/CML511]-2/CML395IR)-B)F2-B-6-5-1-1-B-B	CIMMYT	Line	-	Resistant to major insect pests of maize	CIMMYT (2019)
TL173	[(CML395/CML444)-B-4-1-3-1-B/CML395//DTPWC8F31-1-1-2-2]-5-1-2-2-B*4-B-5-B	CIMMYT	Line	1.83	FAW reaction unknown	Kasoma et al. (2020b)
CZL1310c	[[MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-Sn/GQL5]-B-23-4-1-B*5-B-B-4-B	CIMMYT	Line	0.46	FAW reaction unknown	Kasoma et al. (2020b)
CZL16095	([CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB//INTA-F2-192-2-1-1-1-BBBB]-1-5-1-1-1-BBB-B-B-B*/OFP106)-1-1-5-2-2-B-B-B	CIMMYT	Line	0.52	FAW reaction unknown	Kasoma et al. (2020b)
EBL173782	(CML395IR/([CML395-BB/CML511]-1/CML444IR)-B)F2-B-12-1-2-2-B-B-B-B	CIMMYT	Line	-	FAW reaction unknown	CIMMYT (2019)
Pool 16	MM752 derivative	ZARI	Tester	2.50	OPV, partial/moderate resistant to FAW, with drought escape, moderate resistant to leaf blight, early maturing	Masole and Gumbo (1994); Kasoma et al. (2020b)
ZM 4236	NPGRC	ZARI	Tester		Landrace, relatively resistant to FAW	Kasoma et al. (2020b)
CML545-B	CML545	CIMMYT	Tester	3.50	Relatively resistant to FAW	CIMMYT (2019)
CML346	AC90390SCBSR-F430-1-1-2-3-2-2-B	CIMMYT	Tester	-	Lowland adapted, resistant to FAW and SCB	CIMMYT (2019)
CML334	P590-C3-F374-2-1-2-B-#-3-3-B	CIMMYT	Tester	-	Sub-tropical adapted, early maturing, resistant to FAW and SWCB	CIMMYT (2019)
MM501	MM752 derivative	ZARI	Check	6.00	Single cross, relatively resistant to FAW, suitable for Regions I and II in Zambia, with drought escape, resistant to rust, cob rot and MSV	Masole and Gumbo (1994)
MM502	MM752 derivative	ZARI	Check	7.50	Single cross, relatively resistant to FAW, suitable for Regions II and III, multiple cob production, highly resistant to MSV and leaf blight	Masole and Gumbo (1994)

NPGRC = National Plant Genetic Resources Center (http://www.fao.org/pgrfa-gpa-archive/zmb/nfp.html)/Zambia; ZARI = Zambia Agricultural Research Institute; - = not available; FAW = fall armyworm; SCB = southern corn borer; SWCB = south-western corn borer; MSV = maize streak virus; Regions I, II and III denote agroecological regions in Zambia receiving <750, 750-1000, >1000 mm of rainfall, respectively

6.2.2 Evaluation of experimental hybrids

6.2.2.1 Description of study sites and trial establishment

The study was carried out at Mount Makulu research station (15.5483° S, 28.24817° E, 1227 m elevation), the University of Zambia field station (15.3946° S, 28.3371° E, 1263 m elevation) in Lusaka district and Chiawa (15. 9431° S, 28.9159° E 410 m elevation) in the Chirundu district. Mount Makulu research station and University of Zambia field station are in the agro-ecological Region II of Zambia, characterized by a mean annual rainfall between 750 and 1000 mm and a maximum summer temperature of 32°C. Chiawa belongs to agro-ecological Region I with 400-750 mm annual rainfall, a maximum summer temperature of 37°C (Phiri et al., 2013). The 60 F1 experimental hybrids, 12 parental lines, 5 testers and two hybrid checks were evaluated for FAW resistance at three FAW hotspot sites during the 2019-2020 main crop season (November to April). The experiment at each site was laid out in a 10 × 8 alpha lattice design with two replications. The 79 genotypes were randomly allocated across the eight incomplete blocks consisting of 10 genotypes each, except for the last incomplete block, which contained nine genotypes. The experiments were conducted under rain-fed conditions with supplementary irrigation when necessary. Recommended agronomic practices were conducted according to Mueller and Sisson (2013).

6.2.3 Data collection

The data on agronomic and FAW-related traits were collected following Kasoma et al. (2020b).

6.2.4 Data analyses

6.2.4.1 Analysis of variance

A combined analysis of variance (ANOVA) was conducted following an alpha lattice procedure in Genstat 18th version (Payne, 2017) after testing for normality and homogeneity of variances. The test genotypes were considered as fixed factors, while the sites, replications, and incomplete blocks were treated as random factors.

6.2.4.2 Combining ability estimates

A general linear model (GLM) was used to conduct the combining ability ANOVA in Genstat 18th version (Payne, 2017). The genotypes were considered as fixed effects, while the sites,

replications, and incomplete blocks were treated as random effects. The linear model used to determine the combining ability effects was:

$$Yijk = \mu + Ed + REPk(Ed) + gi + gj + gij + Ed * gij + BLK(REPk) + eij$$

Where *Yijk* is the observed performance of the cross between the i^{th} line, and j^{th} tester; μ is the overall trial mean; *Ed* is the effect of the environment *(d = 1, 2, 3….r) REPk(Ed)* is the effect of replicate *k* nested in environment *d* (*k = 1, 2, 3….m); gi* general combining ability of the lines; *gj* is the general combining ability of the testers; *sij* is the specific combining ability of the cross between line *i* and tester *j*; *BLK(REPk)* random effect of blocks nested in replicate *k; eij* is the residual.

The line × tester model was used to estimate the general and specific combining ability effects of the lines, testers and experimental hybrids. The commercial checks were excluded in the estimation of combining ability effects. The general combining ability effects of the lines and testers, and the specific combining ability of the crosses were calculated according to Singh and Chaudhary (1979). The significance of the estimated GCA effects of the lines and testers, and the SCA effects of experimental hybrids was tested by computing t statistics, based on the method described by Dabholkar (1999).

6.2.4.3 Variance components

Variance components were determined using the restricted maximum likelihood (REML) method, using the Analysis of Genetic Designs with R (AGD-R) software version 5.0 (Rodriguez et al., 2018). The procedure was conducted using the model according to Hallauer (1988), assuming the absence of inbreeding and epistasis. The relative importance of additive and dominance genetic effects were evaluated using Bakers' ratio (Baker, 1978).

The broad (H^2) and narrow sense (h^2) heritability estimates for the measured traits were computed from the estimated variance components as follows:

$$H^2 = (\sigma^2A + \sigma^2D)/\sigma^2T$$

$$h^2 = \sigma^2A/\sigma^2T$$

where σ^2A represents the additive genetic effects; σ^2D represents the dominance genetic effects; and σ^2T is the total variance.

6.2.4.4 Better parent heterosis

Heterosis of the F1 experimental hybrids was assessed against the better performing parent for each trait, according to Sharma, Dhakal et al. (2016), using the following formula:

$$BPH = \left(\frac{F1-BP}{BP}\right) * 100$$

Where BPH is better parent heterosis, F1 is the performance of the experimental hybrid, and BP is the mean value of the better performing parent in a particular cross.
Significance of heterosis was determined using the method outlined by Soehendi and Srinives (2005) as follows;

$$t_c = \frac{\%H-0}{Se}$$

Where t_c is the calculated value, %H is percentage better-parent heterosis (heterobeltosis), and s.e. is the standard error calculated as;

$$se = (2xMSE/r)^{1/2}$$

Where r is the number of replications, MSE is the mean square of the error.

6.3. Results

6.3.1 Analysis of variance

Analysis of variance revealed significant ($p<0.05$) differences among the test genotypes for days to 50% anthesis and silking, plant height, cob placement, the final number of plants, number of cobs, field weight and grain yield . The test genotypes showed highly significant ($p<0.001$) differences for all the FAW parameters (Table 6.2).

6.3.2 Performance of the lines, testers and experimental hybrids for agronomic traits

The performance of lines, testers, experimental hybrids and checks for all the traits measured in the study is presented in Appendix 6.1. Days to 50% anthesis and silking ranged from 53 to 72 and 58 to 73 days, respectively (Table 6.3). The experimental hybrids involving lines TL173, TL13905 and EBL1611480, and the testers Pool16 and CML346 were among the early anthesis and silking hybrids. These included the new hybrids Pool16/TL173, Pool16/TL13905, Pool16/CML548-B, CML346/CZL1310c and CML346/CML548-B, which were among the earliest to flowering and maturity in the study. The experimental hybrids CML346/EBL1611480, CML545-B/EBL169550, CML346/VL050120, CML334/CZL16095 and Pool16/CM545-B with mean plant heights of 135.5, 138.4, 139.0, 143.5 and 144.0 cm were the shortest. The new hybrids with the highest number of cobs per plot were CML346/EBL173782, CML346/EBL16469, Pool16/CMl548-B, CML346/TL151847 and ZM4236/EBL1738809, which all produced more than 8 cobs/plot. About 43% of the experimental hybrids had ear rot damage score of zero. The mean grain yield across all the new hybrids was 0.59 t ha^{-1}. The highest yielding lines producing above 6.0 t ha^{-1} were TL13905, EBL173782, CZL16095 and VL050120. The testers CML346, CML334 and Pool16 were the top three yielders with 6.08, 5.95 and 5.94 t ha^{-1}, respectively. The new hybrids with the highest yields were CML346/EBL16469 (6.70 t ha^{-1}), CML545-B/EBL169550 (6.60 t ha^{-1}), CML346TL13905 (6.54 t ha^{-1}), CML334/EBL169550 (6.51 t ha^{-1}) and ZM4236/EBL173782 (6.47 t ha^{-1}).

The mean days-to-anthesis and silking of the experimental hybrids were comparable to MM501, the earlier maturing of the two checks. On average, the experimental hybrids were taller than both commercial checks. The mean grain yield of some of the new hybrids was higher than that of both checks. Experimental hybrids involving the lines EBL169550, TL13905, CZL1310c, TL173 and EBL16469 and the testers CML334, CML346 and Pool16 performed better than the checks. Notable experimental hybrids included CML334/EBL169550, CML346/TL13905, CML346/EBL16469 and Pool16/TL151487 with grain yields of 6.51, 6.54, 6.70 and 6.4 t ha^{-1}, respectively.

6.3.3 Performance of lines, testers and experimental hybrids for FAW-related traits

The mean values for early- and mid-whorl stage FAW leaf damage (FLD1 and FLD2) were 7.43 and 35.45%, respectively (Table 6.3). The lines TL1512847, EBL1611480, TL173 and

CML548-B had the lowest FAW leaf damage ratings at FLD1, while TL173, CZL1310c, EBL1738809 and TL1512847 had the lowest FAW leaf damage ratings at FLD2. The top performing testers at FLD1 were ZM4236 (6.69%), Pool16 (6.73%) and CML346 (7.08%). At FLD2, the testers Pool16, CML346 and CML545-B had the lowest FAW leaf damage ratings of 23.57, 25.80 and 33.24%, respectively. Mean FAW leaf damage (FLDMean) across the lines, testers, experimental hybrids and checks ranged from 11.72 to 27.77 %. Parental lines TL173, CZL1310c, TL1512847 and EBL1738809 had the lowest mean FLD ratings of 15.54, 15.83, 16.69 and 17.60%, respectively. The top performing testers in terms of mean FLD were Pool16 (15.035%) and CML346 (15.23%). Pool16 maintained low FAW damage ratings for FLD1, FLD2, and FLDMean of 6.73, 23.57, and 15.03%, respectively, which were below the overall mean values. The new hybrids CML545-B/EBL169550, CML346/VL050120, CM334/TL173, Pool16/TL13905 and CML334/CZL16095 exhibited the lowest mean FLD values of 11.72, 13.78, 13.82, 14.27 and 14.29%, respectively. Among the top 10 FAW resistant experimental hybrids, the highest mean grain yield was 6.60 t ha^{-1}, which was attained by CML545-B/EBL169550, followed by 6.17 and 6.11 t ha^{-1} for the hybrids CML334/TL13905 and CMLTL151847, respectively. Over 50% of the experimental hybrids had FCD ratings above the mean. Experimental hybrids derived from the testers CML545-B and CML334, including CML545-B/EBL169550, CML545-B/CZL16095, CML334/VL050120 and CML334/EBL16469, exhibited comparatively low mean FCD ratings (Appendix 6.1).

The performance of the experimental hybrids in terms of FAW leaf damage lies between the performance of the two commercial check hybrids MM501 and MM502.

Table 6.2. Combined analysis of variance and significant tests for agronomic and FAW parameters in maize lines, testers, experimental hybrids and commercial hybrid checks evaluated in three sites

		Agronomic parameters										FAW parameters			
Source of variation	Df	DTA	DTS	ASI	PH (cm)	CP (cm)	FNP	NC	ER	FW (kg)	GY (t ha^{-1})	FLD1 (%)	FLD2 (%)	FLDMean (%)	FCD
Sites	2	5526.25	5155.16	5.02	44043.60	15694.7	231.42	508.20	11.36	2.19	2.51	150.19	14843.63.	279.10	59.46
Sites.Rep	3	25.60	40.43	16.67	2552.40	1594.0	111.43	22.16	0.21	0.01	0.04	41.09	290.06	3.99	0.92
Sites.Rep.Blk	54	53.88	44.27	9.18	1407.80	481.1	23.70	12.61	1.73	0.16	0.22	24.65	310.95	58.32	4.18
Genotypes	78	44.10***	38.07***	8.56	1057.10***	282.0***	29.62***	12.46***	1.12	0.17***	0.20***	7.99***	432.76***	45.64***	3.98***
Genotype.Sites	150	37.27	32.27	8.72	1129.00	236.5	14.54	12.46	1.07	0.13	0.18	6.06	218.76	23.47	3.01
Residual	156	11.66	3.73	8.72	310.50	138.4	8.04	12.13	0.96	0.04	0.06	4.47	40.08	8.53	1.56

*** - significant at 0.001; Site.Rep - site x replication interaction; Site.Rep.Block site x replication x block interaction; Genotypes – lines, testers, experimental hybrids and checks; Genotypes.sites - interaction of the lines, testers, experimental hybrids and checks with the sites; Df - degrees of freedom; DTA- Days to 50% anthesis; DTS - days to 50% silking; ASI - anthesis silking interval; PH - plant height; CP - cob placement; FNP - final number of plants; NC - number of cobs; ER - ear rot;; FW- field weight; GY- grain yield, FLD1- early-whorl stage FAW cob damage; FLD2 - mid-whorl stage FAW cob damage; FLDMean - mean FAW leaf damage; FCD - FAW cob damage

Table 6.3. Mean performance of the top ten and bottom five experimental maize hybrids and the top five lines and testers ranked based on the mean FAW leaf damage rating (FLDMean)

Name	DTA	DTS	ASI	PH (cm)	CP (cm)	NP	NC	ER	FW (kg)	GY (t ha^{-1})	FLD1 (%)	FLD2 (%)	FLDMean (%)	FCD
Top ten experimental hybrids														
CML545-B/EBL169550	70	71	1	138 4	62 27	1 97	1 26	0	5 73	6 60	5 07	17 02	11 72	0
CML346/VL050120	65	66	1	139 0	43 84	3 80	2 93	0	5 71	5 74	6 73	20 90	13 78	2
CML334/TL173	65	66	1	168 5	62 43	8 46	4 06	0	5 89	5 92	6 55	21 08	13 82	1
Pool16/TL13905	57	59	1	153 4	67 77	9 45	4 88	1	5 87	5 89	10 78	27 66	14 27	1
CML334/CZL16095	66	67	1	143 5	67 77	10 48	6 60	1	5 91	5 94	6 07	30 59	14 29	3
CML334/TL13905	59	60	1	168 9	70 02	9 61	5 86	0	6 06	6 17	6 79	25 60	14 38	2
CML334/EBL173782	63	64	1	156 7	71 97	12 55	5 75	0	6 05	6 09	6 84	22 34	14 73	1
CML346/T151847	60	62	2	169 8	70 74	13 74	8 35	0	6 03	6 11	8 20	21 55	14 84	2
CML334/EBL1738809	62	63	2	160 4	51 38	8 52	5 90	0	6 04	6 01	7 27	26 72	14 89	2
ZM4236/VL050120	64	67	2	150 4	54 66	9 94	6 24	1	5 84	5 85	7 00	22 28	15 02	3
Bottom five experimental hybrids														
CML334/CML548-B	62	63	1	156 6	55 09	8 96	6 92	0	5 91	5 97	6 70	54 53	23 44	2
Pool16/EBL1611480	58	59	1	159 7	64 69	13 01	4 00	1	5 90	5 97	8 75	37 62	23 73	3
CML346/CZL1310c	57	60	3	159 9	67 88	10 68	5 61	0	6 10	6 17	6 88	58 99	24 72	2
Pool16/EBL173782	60	62	3	151 2	60 98	12 19	6 70	0	5 87	5 87	9 35	49 05	24 95	3
CML545-B/EBL16469	61	63	2	170 1	70 39	13 05	5 61	0	5 87	5 87	13 05	52 19	27 77	2
Top five lines														
TL173	64	65	2	174 9	62 45	6 44	3 20	0	5 90	5 97	6 25	26 58	15 54	1
CZL1310c	67	69	2	156 8	70 18	8 26	5 53	1	5 93	5 98	7 31	30 06	15 83	1
TL1512847	65	66	1	160 1	63 81	9 40	3 57	0	5 86	5 89	5 52	34 55	16 69	1
EBL1738809	62	63	1	135 6	50 25	10 21	4 00	1	5 76	5 78	7 63	31 55	17 60	1
EBL1611480	63	65	2	181 6	67 21	10 62	3 08	0	5 83	5 86	5 82	38 42	17 97	2
Top five testers														
Pool16	58	59	2	138 2	56 57	7 25	4 55	1	5 88	5 94	6 73	23 57	15 03	2
CML346	62	63	1	168 2	62 46	7 91	5 42	0	5 99	6 08	7 08	25 80	15 23	2
CML545-B	62	63	2	146 0	57 84	7 31	3 80	1	5 81	5 74	7 69	33 24	17 87	1

Table 6.3. Continued

CML334	68	70	1	171 5	71 74	7 66	5 70	1	5 96	5 95	7 20	36 14	18 67	2
ZM4236	67	69	2	154 70	68 56	6 00	3 84	1	5 81	5 81	6 69	41 24	20 50	2
Single cross hybrid checks														
MM501	61	64	3	154 8	60 34	10 64	3 51	1	5 84	5 88	6 34	40 76	20 33	0
MM502	64	66	2	164 5	80 80	8 87	5 10	0	5 87	5 77	78 02	30 12	16 37	1
Statistics														
Experimental hybrid means	62 00	64 00	2 00	166 98	67 34	10 78	6 06	1 00	6 03	6 09	7 56	35 01	18 66	2 00
LSD (0 05)	4 08	2 30	3 35	20 60	13 90	3 30	2 90	1 17	0 25	0 31	2 48	7 41	3 40	1 48
CV (%)	5 49	3 01	162 41	10 74	17 71	27 28	42 48	149 34	40 76	45 51	28 41	17 91	15 67	59 87

LSD - least significant difference; CV- coefficient of variation; DTA- days to 50% anthesis; DTS - days to 50% silking; ASI - anthesis silking interval; PH - plant height; CP - cob placement; NP-number of plants ; NC - number of cobs; ER - ear rot; FW- field weight; GY- grain yield; FLD1 - early whorl stage FAW leaf damage; FLD2 - mid- whorl stage FAW leaf damage; FLDMean - mean FAW leaf damage; FCD - FAW cob damage

6.3.4 Analysis of variance on combining ability effects

The GCA effects of the lines were significant for all measured traits, except for ASI and FLD1 (Table 6.4). The effect of the testers were significantly (<0.01) different for DTA, DTS, FNP, NC, FW, GY, FLD2, FLDMean and FCD. The GCA effects of the lines and testers varied across the test sites, as shown by the significant interaction between the sites with lines and testers for most assessed traits. The SCA effects of the experimental hybrids were highly significant (<0.001) for all measured traits except ASI and ER, as shown by the line × tester × site interaction effects.

Table 6.4. Line-by-tester analysis of variance for agronomic and FAW parameters involving 12 lines, 5 testers and 60 experimental hybrids evaluated in three FAW hotspot areas in Zambia

		Agronomic parameters										FAW Parameters			
Source of variation	DF	DTA	DTS	ASI	PH (cm)	CP (cm)	FNP	NC	ER	FW (kg)	GY (t ha^{-1})	FLD1 (%)	FLD2 (%)	FLDMean (%)	FCD
Site	2	4867.7.24	4484.41	6.62	25845.80	9207.30	258.42	292.46	6.51	1.45	1.71	115.26	9423.04	351.26	35.70
Rep	3	21.01	21.92	17.37	2397.80	1129.20	120.64	37.24	0.18	0.06	0.09	31.67	358.20	5.80	1.51
Block (Rep)	48	38.26	35.63	10.76	1309.30	388.00	32.95	15.73	2.04	0.18	0.27	20.80	314.38	51.63	3.65
Line	11	74.07***	38.16***	12.04	559.80**	413.10**	40.36***	11.26*	2.62***	0.10**	0.12*	6.73	449.95***	47.94***	4.70**
Tester	4	55.09**	79.02***	8.15	118 90	76.70	43.56***	23.76**	0.92	0.24***	0.15*	10.10	1003.57***	97.03***	5.68**
Line × Tester	43	31.51***	25.08***	10.47	1044.50***	301.90***	21.01***	11.67***	1.33**	0.20***	0.25***	10.48***	390.71***	43.00***	4.86***
Line × site	22	61.79***	45.93***	14.51	1245.80***	193.60**	15.62**	14.92***	0.48	0.11***	0.17***	8.21*	148.12***	20.15***	3 25**
Tester × site	8	67.14***	88.50***	4.11	1488.00***	271.2**	11.73	13.34**	1.20	0.25***	0.41***	6.62	272.89***	27.06***	6.78***
Line × tester × site	82	29.07***	20.38***	11.93	900.40***	232.50***	12.76***	10.55***	1.38	0.13***	0.17***	8.57***	203.15***	25.70***	3.23***
Residual	116	14.82	4.29	1045	264 10	117.20	6.76	5.21	0.77	0.03	0.06	4.47	38.52	7.20	1.69

*,**,***- significant at 0.05, 0.01,0.001; respectively; Rep- Replication ; Block (Rep) - Block and replication interaction; Line x Tester - interaction between the lines and testers; Line x site - interaction between the lines and sites; Line x Tester x site - Interaction between the experimental hybrids and the sites ; Df - degrees of freedom; DTA - Days to 50% anthesis; DTS - days; 50% silking; ASI - anthesis silking interval; PH - plant height; CP - cob placement; FNP - final number of plants; NC - number of cobs; ER - ear rot; FW- field weight; GY- grain yield,

6.3.5 General combining ability estimates of lines and testers

Combining ability effects of the lines and testers were variable across the measured traits (Table 6.5). The line CML548-B had a negative and significant GCA effect of -3.89 for DTA in a desirable direction. The lines CML548-B, EBL1738809, EBL1611480, EBL16469 and TL13905 had significant and negative GCA effects for DTS, with lines CML548-B and EBL1611480 contributing to a reduced DTS by nearly two days. However, line CML548-B had the potential to delay the ASI due to its positive GCA effects for this trait. The tester Pool 16, with a GCA estimate of -1.48, had the potential to reduce DTS in a desirable direction. Higher and negative. The GCA effects for FW and GY ranged between -0.16 and 0.11. The line EBL16469 had positive GCA effects for FW of 0.10. The negative GCA effects for FLD and FCD would be desirable for FAW resistance breeding. One line had beneficial GCA effects for FLD1, CZL16095, while lines with beneficial GCA effects for FLD2 were VL050120, EBL1738809 and TL13905. The two former lines also had favourable GCA effects for mean FLD of -2.27 and -1.31. Tester CML334 had a negative and significant GCA estimate for FLD2 of -4.40. Significant and beneficial GCA effect with a value of -0.59 for FCD was shown by line EBL169550 (Table 6.5).

Table 6.5. General combining ability effects of the lines and testers assessed in three FAW hotspot sites in Zambia, based on FAW and agronomic traits

	Agronomic parameters										FAW parameters			
	Lines													
Name	DTA	DTS	ASI	PH	CP	FNP	NC	ER	FW	GY	FLD1	FLD2	FLDMean	FCD
VL050120	3.97***	3.75***	-0.13	-6.15	-3.89	-2.30***	-1.38**	-0.23	-0.09*	-0.11*	-0.71	-8.13***	-2.27***	-0.59*
CML548-B	-3.89***	-1.32**	2.90***	-6.25	-7.90***	-0.22	0.95*	0.78***	-0.04	0.07	-0.60	3.08*	0.54	0.75**
EBL169550	1.20	0.94*	0.42	-4.25	-0.25	-1.67**	-0.29	-0.12	-0.00	0.06	-0.23	-3.76**	-0.52	0.02
TL1512847	1.31	1.02*	-0.20	-3.55	1.72	1.59**	0.60	0.02	0.03	0.03	0.21	1.92	0.50	0.28
EBL1738809	-0.92	-1.04*	-0.02	10.05**	-3.54	-0.05	0.60	-0.41*	0.05	0.00	0.20	-3.59**	-1.31*	-0.40
EBL1611480	-1.39	-1.73***	-0.24	-6.05	-4.59*	0.13	0.32	-0.01	-0.02	-0.04	0.96**	4.05**	1.94**	0.18
EBL16469	-0.52	-0.94*	-0.37	6.55*	5.83**	0.33	0.12	0.00	0.10*	0.07	0.75	1.25	0.83	0.19
TL13905	-0.69	-1.15**	-0.33	-4.25	4.61*	0.08	-0.11	-0.15	0.04	0.04	0.80	-3.72**	-0.84	0.03
TL173	-0.02	-0.26	-0.33	3.25	3.35	1.72**	-0.20	0.22	-0.01	-0.01	-0.65	-1.71	-0.83	-0.39
CZL1310c	0.93	1.04*	0.16	0.95	-2.20	-0.65	-0.43	0.09	-0.04	-0.03	-0.07	4.97***	1.25*	0.32
CZL16095	0.29	0.27*	-0.12	5.05	4.45*	-1.98***	-1.33**	-0.13	-0.14***	-0.16**	-1.08*	2.29	-0.64	-0.43
EBL173782	-0.38	-0.60	-0.28	4.65	2.40	2.28***	1.14*	-0.05	0.04	0.05	0.43	3.32**	1.35*	0.04
	Testers													
Pool16	-1.36	-1.48*	-0.07	-4.80	-0.71	-0.11	0.01	0.12	-0.01	0.00	0.36	0.65	0.86	0.34
CML545-B	1.64	1.54*	-0.07	0.90	-0.93	0.23	-0.61	-0.01	-0.03	0.00	0.02	0.70	0.18	-0.10
ZM4236	0.24	1.05	0.79	2.80	2.31	1.24	0.35	0.03	-0.03	-0.03	-0.56	6.18***	1.24	0.21
CML346	-0.81	-0.98	-0.16	0.50	0.99	0.31	0.88	0.01	0.11*	-0.08	-0.30	-3.14	-1.06	0.02
CML334	0.29	-0.11	-0.49	0.60	-2.37	-1.66*	-0.63	-0.16	-0.04	-0.05	0.48	-4.40*	-1.22	-0.48

*,**,***- significant at 0.05,0.01,0.001, respectively; DTA- days to 50% anthesis; DTS- days to 50% silking;ASI- anthesis silking interval; PH- plant height; CP-cob placement; FNP- final number of plants; NC- number of cobs; ER - ear rot; FW- field weight; GY- grain yield, FLD1- early-whorl stage FAW cob damage; FLD2 - mid-whorl stage FAW cob damage; FLDMean – mean FAW leaf damage; FCD- FAW cob damage

6.3.6 Specific combining ability effects of the experimental hybrids

The specific combining ability effects of the 60 experimental hybrids are presented in Appendix 6.2. The experimental hybrids ZM4236/CML548-B, CML346/CZL1310c Pool16/TL173 and ZM4236/EBL169550 had the only significant and negative SCA estimates of DTA at -5.12, -4.32, -3.91 and -3.11 respectively. Four experimental hybrids had significant and negative SCA estimates for DTS including ZM4236/EBL1611480, ZM4236/EBL169550, Pool16/TL173 and CML346/CZL1310c. The SCA estimates for GY of the new hybrids such as CML334/EBL169550, CML545-B/EBL169550, ZM4236/EBL1611480, and CML346/EBL16469, which ranged between 0.4 and 0.5. The highest, desirable SCA estimate for FLD1 of -2.31 was for the hybrid CML545-B/EBL169550. For FLD2, experimental hybrid CML545-B/EBL169550 had an SCA value of -15.40, followed by CML346/TL151847 (-12.71), and CML334 (-12.06) in a desirable direction. The hybrids reduced FLD2 by more than 12%. For mean FLD, the experimental hybrids with significant and negative SCA effects were CML545-B/EBL169550 (-6.83), CML545-B/CZL1310c (-4.74), Pool16/TL13905 (-4.64) and CML334/EBL173782 (-4.28). For FCD, the experimental hybrid CML545-B/EBL169550 was the only hybrid with a negative and significant SCA effect (-1.86).

6.3.7 Better parent heterosis for agronomic and FAW traits

The assessment of selected experimental hybrids for better parent heterosis (BPH) is presented in Table 6.6. Results showed that some experimental hybrids had reduced DTA (e.g. ZM4236/CML548-B and CML346/CZL1310c) and DTS (e.g. Pool16/TL13905 and CML346/CZL1310c) (Appendix 6.3). The maximum reductions for the two traits were by 8.32 and 6.46%, respectively. Experimental hybrids derived from the lines TL173, CZL1310c, EBL11611480, CML548-B and TL13905 including Pool16/TL173, Pool16/TL13905, ZM4236/EBL1611480, Pool16/EBL1611480, CML334/EBL16469 and CML346/TL13905, flowered and silked considerably earlier than their better parents. Positive and high heterosis for FW and GY are desirable for yield improvement. The new hybrids with the best heterosis for GY were CML346/EBL16469, CML346/EBL169550, ZM4236/EBL1611480, CML334/EBL169550, and CML346/TL13905, attaining heterosis 60% above the better parent for the trait.

Conversely, negative heterosis for FAW-related traits is desirable for resistance breeding. In respect of FAW-related traits, experimental hybrids CML545-B/EBL169550, CML545-B/CZL1310c and CML545-B/VL050120 attained BPH values of -30.85, -23.97, and -21.71%

for FLD1, respectively, than their respective better parents. For FLD2, a decreased BPH of -46.61% was recorded for the new hybrid CML545-B/EBL169550 compared to its better parent. Other new hybrids with favourable BPH for FLD2 included CML334/EBL173782 (-28.12%), CML545-B/CZL1310c (-15.23%) and Pool16 TL13905 (-13.35%). For FLDMean, the highest negative BPH was observed for the experimental hybrids CML545-B/EBL169550 (-36.20%), CML334/TL173 (-21.74%), Pool16/TL13905 (-20.94%), CML334/CZL16095 (-19.08%) and CML334/TL13905 (-18.57%). The BPH of the hybrids for FCD ranged from 0.88 to 86.73%. The experimental hybrids that showed more than 55% reduction in FCD included CML545-B/EBL169550, CML334/VL050120, and CML334/EBL16469 with BPH values of -86.73, -68.70, and -55.69%, respectively.

Table 6.6. Better parent heterosis estimates of the top 10 and bottom five experimental hybrids evaluated for 10 agronomic and four FAW traits at three sites ranked according to FLDMean

Genotype	Agronomic parameters										FAW parameters			
	DTA (%)	DTS (%)	ASI (%)	PH (%)	CP (%)	NP (%)	NC (%)	ER (%)	FW (%)	GY (%)	FLD1 (%)	FLD2 (%)	FLDMean (%)	FCD
Top 10 experimental hybrids														
CML545-B/EBL169550	14.98***	10.08***	-32.89***	-15.09	-6.36	-82 24***	-78.46***	-99.88***	-56.56****	69.62***	-30.85***	-46.61	-36.20***	-86.73***
CML334/TL173	5.02	3.86	-54.42***	0.78	-7.42	-0.33	-33.79***	-0.99	-28.75***	-29.34***	-5.27*	-32.18***	-21.74***	-20.46***
POOL16/TL13905	-4.63	-5.16*	-9.91**	-5.54	1.57	-13.78***	-20.52***	-3.20***	-35.59***	-37.70***	35.46***	-13.35*	-20.94***	-41.55***
CML334/CZL16095	7.36	6.36**	-63.63***	-14 17	-1.01	5.97*	7.68***	47.21***	-17.54***	-19.18***	-6.38**	-1.58	-19.08***	84.37***
CML334/TL13905	-3.25	-3.18	0.07	3.62	3.84	-12 32***	-4.41	-67.34***	-1.21***	-100.00***	-15.95***	-17.63**	-18.57***	30.92***
CML545-B/CZL1310c	6.84	5.55**	-20.62***	9.10	-0.89	2.16	40.89***	89.36***	22.94***	39.24***	-23.97***	-15.23*	-18.31***	8.89***
CML346/VL050120	6.55	5.84**	4.83	-13.72	-30.99**	-65 98***	-46.63***	6.86***	-67.71***	-63.29***	-1.84	-0.24	-17.09***	20.26***
CML346/TL151847	-0.54	-0.50	33.05***	3.73	8.73	10.27***	24.27***	-77.54***	-17.40***	-7.74***	12.30***	-33.36***	-16.72***	-25.45***
CML334/EBL173782	3.50	2.39	-47.85***	-6.28	6.73	-4.64	-20.88***	-7.56***	-3.65***	-8.99***	-14.41***	-28.12***	-16.59***	-15.75***
CML334/EBL1738809	1.82	1.83	4.09	-4.07	-19.57	-21 26***	-12.30***	-44.15***	-6.79***	-13.19***	-6.39**	-14.03*	-15.30***	-6.84***
Bottom five experimental hybrids														
CML545-B/TL173	-0.39	-0.82	73.06***	6.07	3.95	24.31***	2.42	68.13***	-17.26***	-14.95***	-10.35***	62.98***	27.63***	-28.14***
CML334/TL1512847	-2.15	-1.36	9.57**	11.36	3.26	-0.24	-29.96***	-35.02***	-46.11***	-50.35***	28.32***	48.07***	32.39***	15.69***
CML334/CML548-B	7.05	1.88	-15.86***	-2.73	-7.44	-15.87***	-2.12	120.77***	-29.37***	-28.09***	-3.08	75.45***	32.73***	12.01***
CML346/CZL1310c	-5.58	-3.81	107.64***	-4.71	4.33	-4.39	-1.25	-52.52***	-6.66***	1.04***	-5.74**	82.41***	38.72***	5.79***
CML545-B/EBL16469	0.11	0.40	15.97***	1.19	5.85	16.41***	-10.13***	-51.92***	-41.51***	-43.41***	71.36***	44.25***	45.70***	-9.22***
SE	3.58	2.07	3.23	16.25	10.83	2.60	2.28	0.88	0.19	0 25	2.12	6.21	2.68	1.30

*, **, *** - significant at 0.05,0.01,0.001, respectively; SE - standard error; DTA - days to 50% anthesis; DTS - days to 50% silking; ASI - anthesis silking-interval; PH - plant height; CP - cob position; NC - number of cobs; ER - ear rot; FW - field weight; GY - grain yield; FLD1 - earl-whorl stage FAW leaf damage; FLD2 - mid-whorl stage FAW leaf damage; FLDMean - mean FAW leaf damage; FCD - FAW cob damage

6.3.8 Variance components

The variance due to the general combining ability (σ^2gca) was greater than the variance due to specific combining ability (σ^2sca) for DTA, ASI, and ER (Table 6.7). The σ^2sca was comparably larger than the σ^2gca for DTS, CP, FW, and GY, and for all FAW-related traits. The additive genetic variance (σ^2A) values were larger than the dominance genetic variance (σ^2D) for DTA, DTS, ASI, CP, ER, FW, GY, FLD2, and FLDMean. In comparison, FLD1 and FCD had smaller σ^2A than σ^2D. The degree of dominance $(\sigma^2D/\ \sigma^2A)^{1/2}$ was greater than 1.0 for DTA, DTS, ASI, CP, ER, FW, GY, FLD2, and FLDMean, and less than 1.0 for FCD. The broad-sense heritability (H^2) for all the agronomic and FAW-related traits ranged from 0.15 to 0.90. Narrow sense heritability (h^2) for agronomic traits ranged from 0.14 to 0.49. FAW-related traits had narrow-sense heritability estimates of 0.37 to 0.39, while GY had a value of 0.31.

Table 6.7. Variance components and heritability values for agronomic traits and FAW parameters among 60 experimental hybrids derived from a cross of 12 lines × 5 testers when assessed in three FAW hotspot sites in Zambia

	Agronomic parameters							FAW parameters			
Components	DTA	DTS	ASI	CP	ER	FW	GY	FLD1	FLD2	FLDMean	FCD
σ^2site	6.94	6.11	1.79	40.73	0.20	0.03	0.04	1.34	40.48	5.08	0.63
σ^2gcaL	1.04	0.67	0.01	5.95	0.04	0.00	0.00	0.00	0.00	0.00	0.00
σ^2gcaT	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	10.10	0.60	0.00
Total (σ^2gca)	1.04	0.67	0.06	5.95	0.04	0.00	0.00	0.00	10.10	0.60	0.00
σ^2sca	0.91	0.91	0.00	13.22	0.03	0.01	0.01	0.64	43.69	4.28	0.30
σ^2gca/ σ^2sca	1.14	0.74	-	0.45	1.33	0.00	0.00	0.00	0.23	0.14	0.00
σ^2A	7.23	5.75	0.30	72.97	0.29	0.64	0.35	2.55	207.38	19.12	1.19
σ^2D	3.62	3.65	0.02	52.91	0.12	0.05	0.04	2.55	174.75	17.13	1.23
h^2	0.41	0.37	0.14	0.44	0.47	0.37	0.31	0.37	0.49	0.46	0.39
H^2	0.60	0.61	0.15	0.76	0.67	0.76	0.69	0.79	0.90	0.87	0.79

σ^2gcaL - variance of the general combining ability due to the lines; σ^2gcaT - variance of the general combining ability due to the testers; σ^2gca - variance of the general combining ability due to lines and testers; σ^2sca - variance of the specific combining ability due to the experimental hybrids; σ^2site - variance due to the sites; σ^2gca/ σ^2sca - Bakers ratio; σ^2A - additive variance; σ^2D - dominance variance; h^2 - narrow sense heritability; H^2 - Broad sense heritability; DTA- Days to 50% anthesis; DTS - days to 50% silking; ASI – anthesis silking interval; CP - cob placement; ; ER - ear rot; FW- field weight; GY- grain yield, FLD1- early-whorl stage FAW cob damage; FLD2 - mid-whorl stage FAW cob damage; FLDMean – mean FAW leaf damage; FCD- FAW cob damage

6.4 Discussion and conclusion

The genotypes exhibited significant variation for FAW resistance and agronomic performance, which presents opportunities for grain yield and FAW resistance improvement. Some of the new hybrids (e.g. Pool16/TL173, CML346/CZL1310c, CML346/EBL1611480, CML334/VL050120 and CML346/EBL16469) were promising for traits such as DTA, DTS, PH, CP and GY suggesting the complementary nature of the parental lines for hybrid breeding. For instance, early anthesis and silking were recorded in the experimental hybrids such as Pool16/TL173, CML346/CZL1310c, ZM4236/EBL169550 and ZM4236/EBL16116480 attributable to their early-flowering parents including Pool16, CML346 and EBL1611480. Pool16 is an early-maturing and open-pollinated variety (OPV) developed by CIMMYT (Masole and Gumbo, 1994), while CML346 is an early- to medium- maturing CIMMYT inbred line (CML), with resistance to FAW and the southern corn borer (SCB) (*Diatraea crambidoides*) (CIMMYT 2019). Experimental hybrids with high numbers of plants per plot (> 13 plants/plot) such as CML545-B/TL173 (16 plants), ZM4236/EBL16469 (14) and CML346/TL151847 (14), or those that incurred minimal foliar damage such as hybrids CML346/VL050120 (FLDMean = 13.78%), CML334/EBL173782 (14.73%) and Pool16/TL13905 (14.27%) did not necessarily exhibit high ear prolificacy, indicating the negative impact of the insect pest on productivity. In contrast, Rwomushana et al. (2018) reported that most maize farmers in Zambia perceived that FAW was more devastating at the vegetative growth stage compared with the reproductive stage. It would be imperative to identify genotypes with native partial resistance to FAW at both the vegetative and reproductive stages for sustainable and integrated FAW management. Partial resistance is associated with horizontal resistance, a more durable form, suitable for SSA because of the prevelent climatic conditions that support multiple generations of FAW, accelerating pest evolution (Matova et al., 2020).

The narrow range for FLD1 (5.07 to 13.05%) compared to FLD2 (17.02 to 58.99%) ratings (Table 6.3) exhibited by the experimental hybrids could be attributed to the difference in pest occurrence and population pressure, or marked genotype susceptibility at the early versus late growth stages of the crop. The pest population pressure was higher at FLD2 than at FLD1. It was noted that the pattern of FAW damage at FLD1 was not consistent with that at FLD2. However, some experimental hybrids such as CML334/EBL173782, CML545-B/EBL169550 and CML334/EBL16469, exhibited similar damage levels at both FLD1 and FLD2. Conversely, some of the lines and testers that expressed high levels of FAW damage at FLD1

did not show high levels of damage at FLD2. These lines and testers either recovered from FLD1 infestation or had variable susceptibility levels at the early- and mid-whorl maize growth stages corresponding to FLD1 to FLD2, in that order.

Both the GCA effects of the parental lines and testers, and the SCA effects of the experimental hybrids, were significant for most traits, suggesting that trait expression was controlled by additive and dominance gene action in that order. The significance of both additive and dominance gene effects on FAW parameters corroborated previous reports (Williams et al., 1995; Alvarez et al., 2002). The lines CML548-B, EBL173809, EBL169550, VL050120, which exhibited favourable GCA effects for days to 50% anthesis, ear rot, mid-whorl stage FAW leaf damage and mean FAW leaf damage are valuable sources of genes for improving these traits. Based on desirable SCA estimates, experimental hybrids Pool16/TL173, CML334/CZL16095, CML346/EBL16469, CML346/TL151847, CML545-B/CZL1310c and CML545-B/EBL169550 were suitable for improving days to flowering, plant height, grain yield, mid-whorl stage FAW leaf damage, mean FAW leaf damage and FAW cob damage, respectively (Appendix 6.2). The favourable performance of the new hybrids is in agreement with the better-parent heterosis values for the same traits.

This study computed the better-parent heterosis rather than the mid-parent heterosis or heterosis over the commercial hybrid checks due to a lack of released maize cultivars with FAW resistance in SSA (Prasanna et al., 2018). The most favourable heterosis for grain yield was obtained in the experimental hybrids CML346/EBL16469, CML545-B/EBL169550, ZM4236/EBL1611480, CML346/TL13905, CML334/EBL169550 and at 81.37, 69.62, 62.64, 56.88 and 56.86%, respectively (Appendix 6.3). The tester CML545-B had favourable better-parent heterosis for FAW-related traits and grain yield along with CIMMYT inbred lines (e.g. EBL169550 and VL050120). CML545-B yielded 3.9 t ha^{-1} under small-scale farmer conditions and had less damage level by FAW (CIMMYT 2019; Kasoma et al., 2020b). CML334 is an early, sub-tropical inbred line that combined well with CML311, a commonly used inbred line in Zambian released hybrids, while CML346 is a promising lowland adapted inbred line (CIMMYT 2019). Better-parent heterosis values for FCD revealed that experimental hybrids involving the lines TL151742 and TL173, and the testers CLM545-B and CML334 were superior for FAW resistance. Further tests in multiple environments are recommended to validate the level of resistance to FAW foliar or cob damage.

Traits controlled by additive gene action including DTA, ASI, and ER can be improved by recurrent selection methods, which exploit additive variance (Widstrom et al., 1972; Widstrom

et al., 1992). Considering that many SSA adapted cultivars with confirmed earliness to anthesis and silking are available, improvement of these traits is relatively easy (Pswarayi and Vivek, 2007). Conversely, traits including FAW-related parameters and GY found to be controlled by non-additive gene action in the current population can be improved by heterosis breeding (Widstrom et al., 1972; Widstrom et al., 1992). This is essential where there are no validated FAW resistant genotypes and the favourable interaction between susceptible genotypes may result in resistant progeny. For instance, the experimental hybrids Pool16/EBL1611480 and Pool16/EBL173782 had parents with poor GCA effects for mid-whorl stage FAW leaf damage and FAW cob damage resistance, respectively. These parents, however yielded new hybrids with moderate FAW resistance. Similarly, the new hybrids CML334/VL050120 and CML346/CZL16095 were developed from parents with low GCA effects for grain yield, but they provided favourable hybrids with moderate grain yield expression. It is therefore important to establish heterotic groupings of these genotypes in order to select divergent genotypes for developing FAW-resistant and high-yielding cultivars. Previous studies by Widstrom et al. (1972) and Wiseman and Davis (1979) reported that additive effects were more important than non-additive effects in conditioning FAW resistance in maize. The difference could be attributed to the genetic constitution of the test germplasm and the difference in the set of environmental conditions under which the evaluations have been conducted. Studies by Viana and Guimaraes (1997), and Alvarez and Filho (2002) reported equal importance of additive and non-additive genetic effects in conditioning FAW resistance in maize. In the current population, breeding strategies that exploit both additive and non-additive effects can be used for generating desirable breeding populations. Additionally, genomic selection tools can be integrated as novel selection methods for exploiting non-additive gene effects and to accelerate gains in selection (Varona et al., 2018). Trait heritability values were generally higher for FAW-related traits than for the measured agronomic traits (Table 6.7). The moderate heritability estimate for grain yield was expected considering its complex nature of inheritance and the influence of genotype × environment interaction effects (Srdic et al., 2007). Therefore, for grain yield, simultaneous improvement using yield-defining traits such as FW, which is relatively more repeatable and easier to measure, is feasible. Heritability estimates for the FAW traits suggest a greater probability of improving mid-whorl stage FAW leaf damage resistance than both early-whorl stage FAW leaf damage and FAW cob damage resistance. However, breeding efforts should target improvement of both foliar and cob damage resistance to

effectively curb the impact of FAW on maize yield under SSA conditions, where both forms of FAW damage are equally widespread (Prassana et al., 2018).

6.5 References

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6.6 Appendices

Appendix 6.6.1. Mean performance of experimental hybrids, lines, testers and checks evaluated for agronomic and FAW parameters in three sites

	Agronomic Parameters										FAW Parameters			
Genotype	DTA	DTS	ASI	PH (cm)	CP (cm)	NP	NC	ER	FW (kg)	GY (t ha^{-1})	FLD1 (%)	FLD2 (%)	FLDMean (%)	FCD
CML334/CML548-B	62	63	1	156.60	55.09	8.96	6.92	1	5.91	5.97	6.70	54.53	23.44	2
CML334/CZL1310c	63	65	1	171.80	69.88	10.45	6.31	2	5.97	5.92	9.45	32.73	19.69	3
CML334/CZL16095	66	67	1	143.50	66.75	10.48	6.60	1	5.91	5.94	6.07	30.59	14.29	3
CML334/EBL1611480	59	61	2	173.60	62.08	6.79	5.18	1	5.87	5.89	12.50	35.54	22.48	1
CML334/EBL16469	60	61	1	170.10	67.63	4.68	3.67	0	5.97	6.12	6.80	25.11	15.60	1
CML334/EBL169550	66	67	1	170.40	66.90	6.40	5.78	0	6.31	6.51	10.24	22.67	16.09	2
CML334/EBL173782	63	64	1	156.70	71.97	12.55	5.75	0	6.05	6.09	6.84	22.34	14.73	1
CML334/EBL1738809	62	64	2	160.40	51.38	8.52	5.90	0	6.04	6.01	7.27	26.72	14.89	2
CML334/TL13905	59	60	1	168.90	70.02	9.61	5.86	0	6.06	6.17	6.79	25.60	14.38	2
CML334/TL1512847	61	62	1	186.20	69.63	12.43	4.71	0	5.81	5.81	9.98	46.02	23.38	2
CML334/TL173	65	66	1	168.50	62.43	8.46	4.06	0	5.89	5.92	6.55	21.08	13.82	1
CML334/VL050120	63	65	1	175.90	67.85	9.81	4.98	0	6.11	6.17	6.55	23.28	15.17	1
CML346/EBL169550	61	63	2	169.00	73.12	11.98	6.09	1	6.03	6.10	7.72	35.08	19.68	2
CML346/CML548-B	58	60	1	170.90	69.26	10.12	7.20	1	6.18	6.23	6.78	34.32	17.71	2
CML346/CZL1310c	57	60	3	159.90	67.88	10.68	5.61	0	6.10	6.17	6.88	58.99	24.72	2
CML346/CZL16095	59	60	1	177.20	72.59	9.11	6.13	0	6.12	6.13	6.42	29.71	16.84	2
CML346/EBL1611480	65	66	1	135.50	56.78	12.05	7.02	1	5.78	5.80	6.51	38.50	18.34	2
CML346/EBL16469	60	62	2	183.40	79.27	12.03	9.00	1	6.80	6.70	8.15	27.80	17.10	4
CML346/EBL173782	59	61	1	190.60	78.88	14.05	9.64	1	6.15	6.22	7.88	28.62	18.17	3
CML346/EBL1738809	61	63	2	192.90	67.96	10.92	7.79	0	6.04	5.96	8.12	28.71	16.53	2
CML346/TL13905	59	60	1	170.30	72.39	12.65	7.65	1	6.41	6.54	7.49	30.15	18.53	2
CML346/TL151847	60	62	2	169.80	70.74	13.74	8.35	0	6.03	6.11	8.20	21.55	14.84	2
CML346/TL173	63	65	2	160.30	70.54	11.60	5.71	0	6.33	6.30	6.65	26.48	16.42	1
CML346/VL050120	65	66	1	139.00	43.84	3.80	2.93	0	5.71	5.74	6.73	20.90	13.78	2
CML545-B/CML548-B	61	63	3	150.50	51.54	9.37	3.87	2	5.85	5.90	6.35	28.54	16.84	3
CML545-B/CZL1310c	67	68	1	183.40	64.64	11.33	8.01	1	6.12	6.31	5.70	30.67	15.57	2

Appendix 6.6.1 Continued

CML545-B/CZL16095	65	68	3	199.30	76.42	9.51	2.17	1	5.73	5.75	6.67	41.04	18.42	1
CML545-B/EBL1611480	61	62	1	165.30	62.91	10.62	7.35	1	6.10	6.14	7.57	32.24	17.13	3
CML545-B/EBL16469	61	63	2	170.10	70.39	13.05	5.61	0	5.87	5.87	13.05	52.19	27.77	2
CML545-B/EBL169550	70	71	1	138.40	62.27	1.97	1.26	0	5.73	6.60	5.07	17.02	11.72	0
CML545-B/EBL173782	61	62	1	181.20	65.30	13.05	5.86	0	5.99	6.05	9.67	38.44	19.99	2
CML545-B/EBL1738809	60	62	2	163.10	57.12	12.90	6.04	0	6.15	6.24	9.51	40.61	21.99	2
CML545-B/TL13905	63	65	2	155.20	72.71	10.46	6.59	1	6.29	6.36	7.63	28.62	17.45	2
CML545-B/TL1512847	63	65	2	152.70	72.55	11.67	6.94	1	6.14	6.24	6.69	30.71	16.85	4
CML545-B/TL173	61	63	1	178.30	69.13	15.65	6.06	1	5.95	6.01	6.20	55.30	23.05	1
CML545-B/VL050120	68	69	1	167.60	69.95	9.74	4.61	0	5.95	5.95	5.37	32.45	18.79	1
Pool16/CML548-B	58	59	1	144.60	53.87	11.58	8.65	2	6.22	6.33	8.01	29.94	17.37	5
Pool16/CZL1310c	60	63	3	168.70	61.48	6.53	4.47	0	5.94	5.90	7.42	29.50	17.45	2
Pool16/CZL16095	59	62	2	154.80	60.19	9.38	4.03	1	5.87	5.93	6.95	34.43	18.40	1
Pool16/EBL1611480	58	59	1	159.70	64.69	13.01	4.00	1	5.90	5.97	8.75	37.62	23.73	3
Pool16/EBL16469	63	64	1	177.30	76.06	10.94	7.01	1	6.11	6.21	6.61	39.92	18.00	3
Pool16/EBL169550	60	61	1	154.50	54.94	10.23	6.86	1	5.90	5.88	6.59	41.51	22.77	2
Pool16/EBL173782	60	62	3	151.20	60.98	12.19	6.70	0	5.87	5.87	9.35	49.05	24.95	3
Pool16/EBL1738809	60	61	2	187.50	69.18	9.56	5.83	1	6.04	6.08	6.82	30.10	16.98	2
Pool16/TL13905	57	59	1	153.40	67.77	9.45	4.88	1	5.87	5.89	10.78	27.66	14.27	1
Pool16/TL1512847	63	65	2	156.00	65.34	12.83	6.92	1	6.30	6.40	7.57	38.17	19.42	4
Pool16/TL173	56	58	2	172.00	81.97	13.42	7.97	1	6.18	6.32	8.15	32.43	19.15	3
Pool16/VL050120	67	69	2	172.70	84.46	9.46	5.21	0	6.12	6.19	8.62	38.14	20.12	2
ZM4236/CL1310c	65	66	2	160.50	63.57	11.35	4.13	0	5.83	5.93	7.87	47.43	22.13	3
ZM4236/CML548-B	66	64	2	181.00	67.38	12.08	7.38	1	6.16	6.26	6.91	42.69	20.84	3
ZM4236/CZL16095	62	63	1	173.40	81.35	11.12	6.03	1	5.86	5.90	6.11	48.05	21.25	3
ZM4236/EBL1611480	58	59	2	169.60	67.87	12.03	8.00	1	6.38	6.41	7.34	54.15	22.69	2
ZM4236/EBL16469	61	63	2	167.80	71.43	14.02	5.22	0	5.84	5.86	6.52	37.11	19.27	1
ZM4236/EBL169550	60	63	3	174.70	75.51	11.68	7.48	1	6.08	6.15	6.58	34.71	18.30	3

Appendix 6.6.1 Continued

ZM4236/EBL173782	63	65	2	182.10	72.67	13.82	8.01	1	6.30	6.47	6.33	53.66	22.65	2
ZM4236/EBL1738809	63	65	2	182.90	76.35	14.10	8.35	0	6.05	6.05	7.71	30.02	16.61	1
ZM4236/TL13905	66	67	1	166.40	77.01	12.38	5.15	0	5.75	5.73	9.48	45.83	22.40	4
ZM4236/TL1512847	65	67	2	155.30	68.16	11.78	6.67	1	6.05	6.04	6.68	48.41	22.33	1
ZM4236/TL173	64	66	2	175.80	63.75	12.94	5.22	1	5.78	5.84	6.66	33.12	17.21	2
ZM4236/VL050120	64	67	2	150.40	54.66	9.94	6.24	1	5.84	5.85	7.00	22.28	15.02	3
Lines														
CML548-B	66	67	2	133.20	61.45	9.41	4.30	1	5.82	5.84	6.51	45.83	19.74	2
CZL1310c	67	69	2	156.80	70.18	8.26	5.53	1	5.93	5.98	7.31	30.06	15.83	1
CZL16095	65	67	3	148.40	59.00	9.08	4.83	1	5.97	6.07	7.97	43.26	22.41	3
EBL1611480	63	65	2	181.60	67.21	10.62	3.08	0	5.83	5.86	5.82	38.42	17.97	2
EBL16469	66	67	1	141.70	59.66	9.65	4.59	0	6.01	6.01	7.17	43.18	20.80	2
EBL169550	67	68	1	150.80	63.62	9.95	4.34	1	5.95	6.02	6.64	48.95	21.38	1
EBL173782	66	69	2	158.20	59.25	11.20	5.89	0	6.22	6.16	7.38	39.97	20.19	1
EBL1738809	62	63	1	135.60	50.25	10.21	4.37	1	5.76	5.78	7.63	31.55	17.60	1
TL13905	64	66	2	150.20	61.07	11.15	7.73	0	6.19	6.2	8.15	43.13	20.98	2
TL1512847	65	66	1	160.10	63.81	9.40	3.57	0	5.86	5.89	5.52	34.55	16.69	1
TL173	64	65	2	174.90	62.45	6.44	3.20	0	5.90	5.97	6.25	26.58	15.54	1
VL050120	72	73	2	140.80	61.24	9.91	4.58	1	5.99	6.06	7.24	43.37	19.58	2
Testers														
CML334	68	70	1	171.50	71.74	7.66	5.70	1	5.96	5.95	7.20	36.14	18.67	2
CML346	62	63	1	168.20	62.46	7.91	5.42	0	5.99	6.08	7.08	25.80	15.23	2
CML545-B	62	63	2	146.00	57.84	7.31	3.80	1	5.81	5.74	7.69	33.24	17.87	1
Pool16	58	59	2	138.20	56.57	7.25	4.55	1	5.88	5.94	6.73	23.57	15.03	2
ZM4236	67	69	2	154.70	68.56	6.00	3.84	1	5.81	5.81	6.69	41.24	20.50	1
Hybrid checks														
MM501	61	64	3	154.80	60.34	10.64	3.51	0	5.84	5.88	6.34	40.76	20.33	0
MM502	64	66	2	164.50	80.80	8.87	5.10	0	5.87	5.77	8.02	30.12	16.37	1

Appendix 6.6.1 Continued

Statistics														
Mean	62	64	2	163.91	66.30	10.35	5.71	1	6.0	6.06	7.43	35.45	18.64	2.00
LSD (0.05)	4.08	2.30	3.35	20.60	13.90	3.30	2.90	1.17	0.25	0.31	2.48	7.41	3.40	1.48
CV (%)	5.49	3.01	162.41	10.74	17.71	27.28	42.48	149.34	40.76	45.51	28.41	17.91	15.67	59.87
SE	3.42	1.93	2.89	17.62	11.76	2.84	2.47	0.98	0.21	0.25	2.12	6.33	2.92	1.25

LSD - least significant difference; CV - coefficient of variation; DTA - days to 50% anthesis; DTS - days to 50% silking; PH - plant height; CP - cob placement; NP - number of plants; NC - number of cobs; ER - ear rot; FW - fresh weight; GY - grain yield; FLD - early-whorl stage FAW leaf damage; FLD2 - mid-whorl stage FAW leaf damage; FLDMean - mean FAW leaf damage; FCD - FAW cob damage

Appendix 6.6.2. Specific combining ability of the 60 experimental hybrids evaluated for 10 agronomic traits and four FAW-related traits at three sites

Genotype	DTA	DTS	ASI	PH	CP	NP	NC	ER	FW	GY	FLD1	FLD2	FLDMean	FCD
CML545-B/EBL169550	7.36***	4.94**	-0.43	-25.50*	-3.97	-7.45***	-3.96*	-0.56	-0.27*	0.45*	-2.31	-15.4***	-6.83***	-1.86*
CML346/VL050120	0.01	-0.10	0.15	-22.70*	-17.32*	-5.07**	-1.17	0.02	-0.34*	-0.31*	0.14	-3.31	-1.78	0.30
CML334/TL173	2.72**	2.54	-0.81	-2.00	-8.34	-2.46	-1.86	-0.73	-0.12	-0.13	-0.88	-8.29	-3.02	0.03
Pool16/TL13905	-2.10	-2.05	-0.07	-4.80	-3.55	-1.39	-1.14	-0.14	-0.18	-0.24	2.02	-4.75	-4.64*	-1.28
CML334/CZL16095	4.18	3.76*	-1.07	-28.80*	-5.12	2.26	1.82	0.38	0.06	0.06	-0.93	-2.78	-2.74	1.91*
CML334/TL13905	-2.35	-1.88	0.00	5.90	-2.01	0.32	-0.15	-0.20	0.03	0.08	-2.09	-1.76	-2.45	0.52
CML334/EBL173782	1.77	1.60	-0.73	-15.20	2.15	1.06	-1.52	0.02	0.02	-0.01	-1.67	-12.06**	-4.28*	-0.32
CML346/TL151847	-1.64	-1.33	0.58	5.50	3.97	0.98	2.27	-0.55	-0.14	-0.09	0.69	-12.71**	-3.48	-0.85
CML334/EBL1738809	0.73	1.24	0.06	-16.90	-12.5	-0.63	-0.83	0.05	0.00	-0.03	-1.01	-0.77	-1.47	0.29
ZM4236/VL050120	-1.56	-1.64	-0.13	-13.50	-11.18	0.14	1.15	0.62	-0.07	-0.10	0.66	-11.19*	-2.84	1.04
CML334/VL050120	-2.62	-2.40	-0.23	14.80	4.32	2.91	0.24	0.20	0.21	0.24	-0.82	0.33	-0.22	-0.64
CML545-B/CZL1310c	2.55	2.03	-0.55	14.30	0.35	0.89	2.93	0.52	0.16	0.26	-1.85	-10.48*	-4.74*	-0.13
CML334/EBL16469	-1.54	-1.74	-0.56	-3.70	-5.62	-4.86**	-2.57	-0.34	-0.12	0.01	-2.04	-7.22	-2.89	-1.16
CML334/EBL169550	2.74**	2.81*	-0.39	7.40	-0.27	-1.13	-0.05	-0.37	0.32*	0.42*	2.40	-4.65	-1.06	0.20
CML346/TL173	2.32	2.92*	1.04	-10.80	2.14	-1.29	0.43	-0.59	0.18	0.12	0.00	-4.15	-0.57	-1.01
CML346/EBL1738809	0.83	1.13	0.54	15.00	6.45	-0.20	1.71	0.01	-0.15	-0.20	0.63	-0.04	0.02	0.57
ZM4236/EBL1738809	2.36	1.67	-0.75	2.80	10.16	2.05	1.27	0.02	0.00	-0.01	0.48	-7.99	-2.21	-0.91
CML346/CZL16095	-1.63	-2.37	-0.42	4.30	3.09	-1.08	1.99	-0.55	0.12	0.13	0.2	-4.92	-0.34	0.03
CML545-B/CML548-B	1.15	-0.37	-1.24	-11.40	-7.05	-1.50	-2.59	0.19	-0.20	-0.25	-0.61	-10.72*	-2.76	-0.28
CML545-B/TL1512847	-1.27	-1.2	-0.03	-11.90	4.34	-1.01	0.83	0.16	0.11	0.12	-1.14	-7.39	-2.70	1.57
Pool16/EBL1738809	0.46	0.49	-0.05	15.00	6.01	-1.14	-0.90	0.27	-0.02	-0.01	-1.33	-2.44	-1.45	-0.50
CML346/EBL16469	-0.31	0.09	0.71	9.00	8.39	0.52	3.40*	0.27	0.56***	0.47*	0.09	-5.79	-1.55	1.98*
CML545-B/EBL1611480	-1.12	-1.16	-0.16	3.20	1.01	-0.34	1.51	0.34	0.11	0.10	-1.01	-7.99	-3.87*	1.16
ZM4236/TL173	2.18	1.61	-0.44	2.50	-9.33	-0.88	-1.04	-0.13	-0.24	-0.23	0.27	-6.77	-2.09	-0.07
Pool16/CML548-B	1.46	-1.48	-2.68	-11.60	-4.94	1.05	1.58	0.81	0.17	0.18	0.71	-9.27*	-2.91	1.70
CML545-B/TL13905	0.22	1.31	0.92	-8.70	1.61	-0.72	1.18	0.15	0.26*	0.24	-0.79	-3.84	-0.77	-0.15
Pool16/CZL1310c	-1.64	-0.45	1.12	5.30	-3.03	-3.57*	-1.22	-0.56	-0.03	-0.16	-0.46	-11.6**	-3.54	-0.66

Appendix 6.6.2 Continued

CML346/CML548-B	0.84	-1.42	-2.08	9.30	12.11	-0.83	0.77	-0.34	0.00	0.00	0.13	-1.10	-0.65	-1.14
POOL16/EBL16469	3.12**	3.01*	-0.17	8.30	3.52	-0.15	0.77	-0.17	0.00	0.05	-2.10	2.54	-2.57	0.05
CML346/EBL173782	-1.05	-0.91	0.29	18.10	11.43	0.59	3.01	0.70	-0.03	0.00	0.15	-7.04	-0.99	0.40
ZM4236/EBL169550	-3.11**	-2.79*	0.4	8.90	6.03	1.25	1.30	0.06	0.07	0.04	-0.23	-3.13	-3.76*	0.59
CML346/EBL1611480	5.13*	5.17***	0.25	-26.30*	-3.68	1.01	1.21	-0.18	-0.34*	-0.33*	-1.75	2.11	-1.42	0.04
Pool16/CZL16095	-1.67	-0.40	0.88	-12.70	-10.97	-0.39	-0.77	0.32	0.00	0.01	0.08	-3.99	-0.70	-0.98
CML545-B/CZL16095	1.61	2.74	1.23	26.10*	5.48	-0.60	-2.01	-0.02	-0.14	-0.18	0.13	2.57	0.00	-0.88
CML346/TL13905	-0.89	-1.15	-0.07	6.70	2.73	1.39	2.28	0.41	0.23	0.33*	-0.62	1.53	1.55	-0.45
CML545-B/VL050120	0.89	0.72	-0.25	5.60	7.35	0.95	0.47	-0.28	0.04	-0.03	-1.54	4.40	2.00	-0.53
Pool16/TL173	-3.91**	-3.74*	0.26	6.30	11.91	0.95	2.04	0.08	0.16	0.22	0.84	-1.99	0.24	0.76
ZM4236/EBL16469	-0.22	-0.4	-0.54	-8.80	-4.13	2.48*	-1.37	-0.32	-0.27*	-0.28	-1.27	-5.74	-1.68	-1.46
Pool16/TL1512847	1.66	1.98	0.23	-2.90	-3.09	0.49	0.19	0.62	0.26*	0.28*	-0.60	0.12	-0.81	0.74
CML346/EBL169550	-1.07	-0.60	0.91	5.40	8.32	1.58	0.90	0.44	-0.11	-0.12	0.66	6.50	-0.07	-0.26
CML334/CZL1310c	0.38	0.57	-0.55	3.60	4.66	1.90	0.62	1.18*	0.02	-0.09	1.44	-3.32	0.79	0.92
CML545-B/EBL173782	-2.17	-2.37	-0.11	8.40	-3.59	-0.33	-0.80	-0.61	-0.05	-0.09	1.62	-1.06	-0.41	-0.38
Pool16/VL050120	2.63	3.11*	0.33	16.40	21.64**	1.01	0.46	-0.57	0.21	0.21	1.37	10.14*	2.65	-0.02
ZM4236/CML548-B	-5.12**	1.01	6.13**	17.20	5.55	0.20	-0.03	-0.54	0.12	0.14	0.52	-1.99	0.18	0.15
ZM4236/CZL16095	-0.52	-2.00	-1.33	-1.70	7.17	0.00	0.89	0.06	0.00	0.01	0.15	4.16	1.77	0.71
CML545-B/EBL1738809	-2.25	-1.86	0.27	-15.10	-5.83	1.86	-0.08	-0.11	0.10	0.16	1.70	8.02	4.24*	0.71
ZM4236/CL1310c	2.08	0.92	-1.15	-10.50	-3.96	-0.10	-1.91	-0.63	-0.13	-0.10	0.90	0.86	0.77	-0.05
ZM4236/TL1512847	2.19	1.88	-0.32	-11.20	-3.29	-1.91	-0.40	-0.05	0.02	-0.05	-0.57	4.89	1.72	-1.53
ZM4236/TL13905	4.51**	3.76*	-0.79	0.60	2.67	0.19	-1.21	-0.09	-0.30*	-0.37*	1.64	7.95	3.12	1.36
CML334/EBL1611480	-1.59	-0.38	0.75	12.40	-0.75	-2.28	-1.26	0.18	-0.11	-0.11	3.46*	0.41	2.89	-0.61
ZM4236/EBL173782	1.74	0.84	-0.81	7.40	0.54	-0.57	0.39	0.46	0.26*	0.36*	-1.14	8.74*	1.19	-0.04
ZM4236/EBL1611480	-2.71	-3.45*	-0.79	5.60	2.73	0.06	1.21	-0.07	0.40**	0.39*	-0.66	8.50*	0.64	-0.24
Pool16/EBL169550	-1.39	-2.08	-0.78	-3.70	-11.52	1.15	1.02	-0.04	-0.10	-0.26	-1.13	9.14*	1.10	-0.10
CML545-B/TL173	-1.96	-2.07	-0.27	6.90	-0.71	2.84*	0.75	0.24	-0.06	-0.09	-0.77	20.83***	4.82*	-0.42
CML334/TL1512847	-1.72	-1.88	-0.61	22.50*	0.49	1.64	-2.01	-0.20	-0.22	-0.26	1.69	13.02**	5.23**	-0.01
CML334/CML548-B	3.70**	1.27	-2.49	-4.40	-4.43	-0.02	-0.15	-0.16	-0.13	-0.13	-0.73	20.37***	5.25**	-0.54

Appendix 6.6.2 Continued

Pool16/EBL1611480	-0.88	-1.14	-0.38	3.30	2.57	2.39	-2.46	-0.29	-0.09	-0.08	-0.17	-2.56	2.06	-0.24
CML346/CZL1310c	-4.32**	-3.42*	1.18	-8.90	5.03	0.16	0.57	-0.45	0.00	0.03	-0.34	21.68***	5.66**	-0.19
Pool16/EBL173782	0.08	1.02	1.04	-15.90	-8.13	-0.85	-0.58	-0.74	-0.18	-0.27	0.97	9.60*	3.87*	0.22
CML545-B/EBL16469	-1.65	-1.30	0.30	-4.60	-1.93	1.62	-0.03	-0.35	-0.23	-0.28*	4.68**	14.76**	7.89***	-0.39

*, **, *** - significant at 0.05, 0.01, 0.001, respectively; DTA - days to 50% anthesis; DTS - days to 50% silking; ASI - anthesis silking-interval; PH - plant height; CP - cob position; NP - Number of plants; NC - number of cobs; ER - ear rot; FW - field weight; GY - grain yield; FLD1 - early-whorl stage FAW leaf damage; FLD2 - mid-whorl stage FAW leaf damage; FLDMean - mean FAW leaf damage; FCD - FAW cob damage

Appendix 6.6.3. Better parent heterosis estimates of the 60 experimental hybrids evaluated for 10 agronomic and four FAW traits at three sites

	Agronomic Parameters										FAW Parameters			
Genotype	DTA (%)	DTS (%)	ASI (%)	PH (%)	CP (%)	NP (%)	NC (%)	ER (%)	FW (%)	GY (%)	FLD1 (%)	FLD2 (%)	FLDMean (%)	FCD (%)
CML545-B/EBL169550	14 98***	10 08***	-32 89***	-15 09	-6 36	-82 24***	-78 46***	-99 88***	-56 56***	69 62***	-30 85***	-46 61	-36 20***	-86 73***
CML346/VL050120	6 55***	5 84**	4 83	-13 72	-30 99**	-65 98***	-46 63***	6 86***	-67 71***	-63 29***	-1 84	-0 24	-17 09***	20 26***
CML334/TL173	5 02	3 86	-54 42***	0 78	-7 42	-0 33	-33 79***	-0 99	-28 75***	-29 34***	-5 27*	-32 18***	-21 74***	-20 46***
Pool16/TL13905	-4 63	-5 16*	-9 91**	-5 54	1 57	-13 78***	-20 52***	-3 20***	-35 59***	-37 70***	35 46***	-13 35*	-20 94***	-41 55***
CML334/CZL16095	7 36***	6 36**	-63 63***	-14 17	-1 01	5 97*	7 68***	47 21***	-17 54***	-19 18***	-6 38**	-1 58	-19 08***	84 37***
CML334/TL13905	-3 25	-3 18	0 07	3 62	3 84	-12 32***	-4 41	-67 34***	-1 21***	-100 00***	-15 95***	-17 63**	-18 57***	30 92***
CML334/EBL173782	3 50	2 39	-47 85***	-6 28	6 73	-4 64	-20 88***	-7 56***	-3 65***	-8 99***	-14 41***	-28 12***	-16 59***	-15 75***
CML346/TL151847	-0 54	-0 50	33 05***	3 73	8 73	10 27***	24 27***	-77 54***	-17 40***	-7 74***	12 30***	-33 36***	-16 72***	-25 45***
CML334/EBL1738809	1 82	1 83	4 09	-4 07	-19 57	-21 26***	-12 30***	-44 15***	-6 79***	-13 19***	-6 39**	-14 03*	-15 30***	-6 84***
ZM4236/VL050120	3 90	3 27	39 68***	-6 64	-13 96	-17 85***	-3 75	145 84***	-32 86***	-37 33***	2 04	-19 01**	-9 63***	77 53***
CML334/VL050120	2 18	2 13	-13 38***	9 19	6 80	6 63*	-18 73***	8 09***	24 08***	24 45***	-4 46*	-15 38*	-8 72**	-68 70***
CML545-B/CZL1310c	6 84***	5 55**	-20 62***	9 10	-0 89	2 16	40 89***	89 36***	22 94***	39 24***	-23 97***	-15 23*	-18 31***	8 89***
CML334/EBL16469	-1 92	-2 94	-39 17***	1 73	0 30	-58 25***	-41 22***	-66 58***	-25 71***	-6 00***	-15 87***	-19 21**	-11 66***	-55 69***
CML334/EBL169550	6 37	5 92**	-25 78***	4 54	-0 79	-30 43***	-5 71*	-95 91***	51 56***	56 86***	39 75***	-27 06***	-8 89***	12 87***
CML346/TL173	3 78	4 26*	58 85***	-4 47	8 42	-7 86**	-3 53	-53 42***	29 81***	20 44***	-3 79	-18 12**	-7 86**	-53 72***
CML346/EBL1738809	0 17	0 26	43 57***	14 96	6 39	-2 24	15 90***	7 96***	-15 15***	-31 18***	11 24***	-10 42	-5 97*	32 74***
ZM4236/EBL1738809	4 42	4 38*	2 18	7 59	19 52	16 53***	24 16***	19 89***	-5 57***	-6 57***	9 56***	-6 33	-5 52*	-38 01***
CML346/CZL16095	-2 20	-3 36	-39 20***	5 60	11 57	-18 44***	11 69***	-97 40***	-3 68***	-4 89***	-12 04***	-8 13	-5 50*	3 40**
CML545-B/CML548-B	4 97	1 90	56 58***	-6 52	-13 41	-15 51***	-45 23***	144 44***	-40 20***	-38 90***	-16 66***	-21 12***	-11 65***	22 26***
CML545-B/TL1512847	0 72	0 54	-6 32	-6 72	9 10	-6 34*	3 35	26 42***	13 25***	18 03***	-12 16***	-15 12*	-11 59***	86 87***
Pool16/EBL1738809	-0 76	-0 89	-2 06	15 46	8 30	-11 65***	-13 27***	146 11***	-8 05***	-1 45***	-12 16***	-6 08	-3 41	-8 51***
CML346/EBL16469	-1 37	-1 36	29 85***	9 30	21 84**	7 31**	44 35***	41 80***	103 36***	81 37***	11 60***	-14 04*	-4 04	96 70***
CML545-B/EBL1611480	1 00	0 63	-14 21***	2 54	0 13	-4 24	14 03***	0 50	16 09***	10 14***	-0 68	-10 89	-10 13***	62 92***
ZM4236/TL173	4 07	4 23*	23 70***	3 41	-8 59	2 78	-19 41***	12 34***	-49 09***	-42 81***	-3 62	-2 39	-4 71	8 31***
Pool16/CML548-B	0 31	-4 52*	-28 14***	-10 19	-9 49	7 72**	22 47***	198 50***	24 99***	27 60***	15 89***	-17 13**	-10 56***	95 63***
CML545-B/TL13905	3 19	4 59*	57 45***	-4 79	9 34	-5 68*	9 57***	26 85***	38 70***	36 60***	0 17	-10 34	-3 32	-5 79***

Appendix 6.6.3 Continued

Pool16/CZL1310c	-1 18	0 95	78 29***	3 88	-5 73	-39 26***	-27 13***	-56 55***	-13 89***	-31 81***	-0 99	-18 35**	-11 60***	-12 51***
CML346/CML548-B	0 19	-3 85	10 13**	1 85	16 36	-9 40***	1 85	63 35***	6 59***	10 27***	-1 90	6 12	-0 62	-17 02***
Pool16/EBL16469	4 32	3 34	-16 94***	9 17	14 0	-2 41	12 44***	-7 83***	-4 02***	7 52***	-16 87***	10 49	-8 68**	16 64***
CML346/ EBL173782	-2 35	-2 42	3 74	13 59	21 24	6 76**	32 70***	114 10***	1 62***	8 33***	7 94***	-11 5	1 96	19 70***
ZM4236/EBL169550	-3 09	-2 69	79 47***	7 18	12 42	-3 47***	15 35***	15 93***	8 27***	1 04***	-6 62**	8 88	-9 05***	36 16***
CML346/EBL1611480	7 32***	6 81**	4 44	-15 94	-9 63	7 88**	8 93***	-25 38***	-55 60***	-54 96***	-10 85***	19 05**	2 92	9 89***
Pool16/CZL16095	-2 29	-0 21	47 75***	-4 68	-9 79	-12 74***	-34 38	80 98***	-27 22***	-26 77***	7 20***	-4 71	0 82	-35 49***
CML545-B/CZL16095	5 39	6 73**	69 14***	18 56	14 92	-14 25***	-60 72***	-5 19***	-55 23***	-58 06***	2 81***	13 43*	0 93	-54 43***
CML346/TL13905	-2 60	-3 40	-27 59***	4 48	11 27	13 25***	27 27***	79 81***	42 21***	56 88***	2 56	-5 55	3 98	-18 75***
CML545-B/VL050120	7 69*	6 88**	-19 04***	4 03	10 11	-12 17***	-16 64***	-63 91***	-10 51***	-23 18***	-21 71***	17 96**	13 06***	-38 52***
Pool16 /TL173	-6 52**	-6 46**	12 49***	5 91	22 86*	6 59*	29 74***	37 13***	23 57***	36 84***	17 87***	-4 42	6 04*	60 49***
ZM4236/EBL16469	0 16	1 06	17 64***	-1 29	2 42	15 87***	-19 52***	-42 27***	-46 85***	-46 29***	-7 40***	0 60	-2 23	-51 54***
Pool16/TL1512847	4 93	4 84*	9 41**	-3 94	-2 07	2 97	2 95	105 86***	41 59***	45 14***	-2 64	5 65	0 21	43 51***
CML346/169550	0 21	0 54	50 66***	3 68	12 39	7 25**	4 53*	79 55***	-17 42	-9 10***	5 77**	10 04	10 44***	-10 45***
CML334/CZL1310c	2 12	2 54	-19 87***	2 75	7 15	2 25	2 85	247 53***	-5 45***	-25 08***	26 11***	5 31	11 49***	71 15***
CML545-B/EBL173782	-0 74	-1 31	-11 81***	7 79	-1 80	-0 84	-19 40***	-98 42***	-14 38***	-14 14***	26 98***	6 25	4 88	-15 81***
Pool16/VL050120	10 96**	11 07***	15 23***	7 20	32 95**	-12 00***	-15 23***	-101 05***	22 86***	16 84***	25 66***	38 64***	21 06***	194 20***
ZM4236/CML548-B	-8 32*	3 33	325 24***	12 42	13 21	-0 17	4 50	33 89***	15 02***	16 85***	-0 03	10 25	7 31**	36 92***
ZM4236/CZL16095	-0 32	-1 48	-31 81***	2 00	16 65	-8 10***	-7 05**	17 63***	-28 16***	-28 31***	-5 74**	26 68***	16 44***	51 92***
CML545-B/EBL1738809	-0 87	-0 50	17 16***	-2 97	-10 58	16 32***	-10 22***	-41 67***	12 18***	26 32***	24 86***	26 71***	25 09***	34 18***
ZM4236/CL1310c	4 87	3 07	-10 26***	-4 58	-2 53	-6 20*	-36 33***	-76 49***	-34 68***	-22 96***	11 70***	16 79**	9 99***	10 84***
ZM4236/TL1512847	5 66	4 56*	4 95	-5 13	-1 42	-5 46***	-0 74	-2 74**	-2 72***	-13 43***	-5 07*	28 89***	15 22***	-51 25***
ZM4236/TL13905	7 94***	7 74***	19 33***	2 09	10 42	2 31	-20 49***	-11 18***	-57 06***	-63 44***	34 64***	43 58***	24 10***	70 54***
CML334/ EBL1611480	-2 03	-0 78	12 28***	7 69	-1 19	-36 78***	-19 62***	32 50***	-29 14***	-29 59***	54 71***	14 35*	27 29***	-24 43***
ZM4236/EBL173782	3 37	3 02	15 51***	7 12	4 20	5 02	10 23***	78 80***	39 30***	50 58***	-10 10***	37 73***	12 57***	7 70***
ZM4236/EBL1611480	-3 98	-3 87	-18 59***	5 21	8 02	-0 58	23 46***	-5 46***	70 78***	62 64***	4 22*	[redacted]	12 77***	-0 88
Pool16/EBL169550	-0 32	-1 84	17 58***	-4 86	-17 66	-4 84	11 73***	13 73***	-24 61***	-40 91***	-10 11***	30 21***	15 35***	10 94***
CML545-B/TL173	-0 39	-0 82	73 06***	6 07	3 95	24 31***	2 42	68 13***	-17 26***	-14 95***	-10 35***	62 98***	27 63***	-28 14***
CML334/TL1512847	-2 15	-1 36	9 57**	11 36	3 26	-0 24	-29 96***	-35 02***	-46 11***	-50 35***	28 32***	48 07***	32 39***	15 69***

Appendix 6.6.3 Continued

CML334/CML548-B	7 05***	1 88	-15 86***	-2 73	-7 44	-15 87***	-2 12	120 77***	-29 37***	-28 09***	-3 08***	75 45***	32 73***	12 01***
Pool16/EBL1611480	-3 59	-4 23*	-28 81***	-0 93	2 96	21 02***	-37 99***	-24 71***	-22 45***	-19 79***	10 01***	4 12	20 21***	4 50***
CML346/CZL1310c	-5 58	-3 81	107 64***	-4 71	4 33	-4 39	-1 25	-52 52***	-6 66***	1 04***	-5 74**	82 41***	38 72***	5 79***
Pool16/EBL173782	-0 50	0 68	63 32***	-6 90	-8 6	-7 37**	-7 79***	-98 37***	-36 31***	-41 91***	17 55***	35 76***	26 39***	25 14***
CML545-B/EBL16469	0 11	0 40	15 97***	1 19	5 85	16 41***	-10 13***	-51 92***	-41 51***	-43 41***	71 36***	44 25***	45 70***	-9 22***
SE	3 85	2 07	3 23	16 25	10 83	2 60	2 28	0 88	0 19	0 25	2 12	6 21	2 68	1 30

*, **, *** - significant at 0.05,0.01,0.001, respectively; SE - standard error; DTA - days to 50% anthesis; DTS - days to 50% silking; ASI- anthesis silking-interval; PH - plant height; CP - cob position; NC - number of cobs; ER - ear rot; FW - field weight; GY - grain yield; FLD1 - earl-whorl stage FAW leaf damage; FLD2 - mid-whorl stage FAW leaf damage; FLDMean - mean FAW leaf damage; FCD - FAW cob damage

OVERVIEW AND IMPLICATIONS OF THE STUDY

Introduction

The arrival of the fall armyworm (FAW) in sub-Saharan Africa (SSA), including Zambia, threatens food security. The pest causes severe yield loss or crop failure in the major cereal crops, especially the main cereal of the region, maize. An integrated FAW control approach emphasizing host-plant resistance has been identified as the most economic and sustainable method of control. However, maize varieties that are resistant to FAW have not yet been bred and deployed in SSA. Breeding for FAW resistance requires knowledge of the pest biology and development, rearing and multiplication protocols for the pest, to facilitate controlled screening, and selection of farmer-preferred and locally-adapted, high yielding maize varieties, among others. Therefore, the overall aim of this study was to develop FAW resistant, farmer-preferred and locally adapted maize genotypes for breeding. This chapter highlights the objectives of the overall project, the major findings of the research together with the implications these have for FAW resistance breeding in maize. Ideas on future research and cultivar development incorporating FAW resistance are presented as recommendations.

The primary objectives of the study were:

i. To identify current constraints to maize production and to assess the impact of the recent FAW outbreak in various districts in Zambia, as a guide to breeding for resistance to FAW;
ii. To screen diverse maize germplasm and select promising genotypes with superior levels of FAW resistance, yield, and yield-related traits for breeding;
iii. To investigate the biology and development of FAW; to develop a standardised methodology for laboratory rearing of FAW, and to evaluate the efficacy of artificial inoculation of FAW using selected maize genotypes for the efficient identification of FAW resistant maize genotypes under controlled conditions for resistance breeding;
iv. To determine the genetic diversity of 59 maize genotypes of diverse genetic background with variable resistance to fall armyworm, using phenotypic traits and SNP-based DArT markers;
v. To investigate the combining ability effects and trait inheritance among the selected maize genotypes, and to select desirable parents and single-cross hybrids with partial FAW resistance and suitable agronomic traits for effective FAW resistance in pre-breeding and breeding programs of maize in Zambia and related agro-ecologies.

Summary of the major findings and implications for maize breeding and production

Farmers' perceptions of production constraints and their trait preferences in maize: implications on breeding for FAW resistance in Zambia

A participatory rural appraisal (PRA) study was conducted in two FAW affected districts in Zambia using semi-structured questionnaires, preference ranking and focus group discussions. The core findings of the study were:

- High costs of fertilizers, limited availability of agricultural land, insect pests, and drought stress were reported by 73%, 55%, 38% and 36.6% of the respondents, respectively, as the main production constraints.
- There were significant differences (X^2 = 12.415; p = 0.002) in the severity of FAW infestation between the surveyed districts in 2016, while the differences were non-significant in 2017.
- FAW resistance should be considered a key trait to be screened for in maize, in addition to other traits such as drought tolerance, in maize cultivar development and deployment in Zambia.
- Data presented in this study will serve as a basis for the breeding and release of farmer-preferred maize varieties, integrating FAW resistance, in Zambia or related agro-ecologies in SSA.

Screening of inbred lines of tropical maize for resistance to FAW, and for yield and yield-related traits

Two sets of diverse maize germplasm were screened for FAW resistance, and yield and yield related traits in a FAW hotspot area in Zambia. Set I and II contained 60 and 253 tropical maize genotypes, respectively. The key results were:

- Highly significant differences ($p < 0.001$) were detected among the test genotypes for FAW-leaf damage (FLD), FAW cob damage (FCD) and agronomic traits.
- Five principal components (PCs) accounted for ≥80% of the total variation. These incorporated important traits including reduced anthesis-silking interval, plant height, FAW leaf and cob damage, desirable ear aspect and grain yield.
- Negative correlations were recorded between grain yield and mean FAW leaf damage ($r = -0.18$, $p < 0.05$), and FAW cob damage ($r = -0.15$, $p < 0.05$).

- Promising genotypes with partial FAW resistance, including CZL1310c, CML444-B, CZL15220, TL1512847 and CML491, were selected for their low mean FAW leaf damage, FAW cob damage, earliness to flowering and high grain yield. These genotypes are recommended as parental genotypes for breeding.

Screening for FAW resistance in maize: methodologies for controlled evaluation and genotype selection

Field-collected FAW egg masses and larvae were used to initiate fresh colonies of larvae and eggs for laboratory rearing of the FAW. Laboratory-reared FAW were used to infest maize test genotypes selected for evaluation for FAW leaf-damage resistance. The main findings were:

- An improved understanding of the salient features of FAW growth and development under local environments as a foundation on which to build integrated FAW management strategies;
- Inoculated maize genotypes revealed differential FAW reaction types, suggesting the test conditions applied were useful as a screening tool.
- The genotypes TL13159, TL02562, TL142151, VL050120, and CML548-B expressed strong resistance reactions to the FAW, while genotypes CML545-B, CZL1310c, CZL16095, EBL169550, ZM4236 and Pool 16 expressed moderate resistance;
- A standardised laboratory and screenhouse-based protocol for the mass rearing and artificial infestation of FAW was developed as a tool to screen maize genotypes for resistance breeding programs in Zambia or other sub-Saharan Africa countries.

The ease of FAW rearing under SSA conditions indicates that the establishment of quality insect-rearing and resistance screening facilities can strengthen research on the management of FAW by enabling simultaneous generation and validation of screening trial results.

Revealing the genetic diversity of maize (Zea mays L.) populations by phenotypic traits and DArTseq markers for variable resistance to FAW

A set of 59 diverse maize genotypes were profiled using agro-morphological traits, FAW damage parameters, and Diversity Array Technology Sequencing-derived single nucleotide polymorphism (SNP) markers. The main results were:

- Significant ($p < 0.001$) differences were observed among the genotypes for 13 phenotypic traits with phenotypic coefficients of variation ranging from 2.19 to 51.79%;

- There was notable phenotypic variation for ear position, grain yield, FAW induced leaf and cob damage;
- Moderate genetic variation was revealed by single nucleotide polymorphisms, with greater variation within rather than among maize test populations;
- Two main clusters were detected in the test populations. This resulted in the identification of three divergent open pollinated varieties (OPVs) including Pool 16, ZM 4236 and ZM 7114, with favourable agronomic performance and FAW resistance. These are recommended for further breeding

Combining ability of maize genotypes for FAW resistance, yield and yield-related traits

This study involved 17 parental genotypes consisting of 12 lines and five testers selected for FAW resistance and favourable agronomic traits. These were crossed using a line × tester mating design and 60 experimental maize hybrids were field-evaluated in three FAW hotspot locations in Zambia. The core findings of the study were:

- There were significant ($p < 0.05$) effects for both the general and specific combining ability for assessed agro-morphological and FAW-related traits;
- Non-additive genetic effects were more important than additive effects for the inheritance of grain yield and FAW-inflicted leaf and cob damage;
- The study selected promising experimental hybrids including CML346/EBL16469, ZM4236/CML545-B, CML346/CZL1310c, CML334/EBL173782, CML545-B/EBL169550. These hybrids have favourable specific combining ability effects for assessed agro-morphological and FAW-related traits. The experimental single cross candidates are recommended for direct production or three-way hybrid breeding in Zambia.

Implications of the findings of this study for FAW resistance breeding in maize

Participatory Rural Appraisal

In order to design effective pre-breeding and breeding programs that are characterised by increased adoption of novel varieties, timely and regular interactions between breeders and farmers are essential. Farmer-breeder interactions that foster bottom-up, fast-evolving methods for sharing, enhancing and analysing farmers' knowledge of their agricultural livelihoods and conditions are important for designing effective breeding programs. The identification and ranking of the key maize production constraints in this study provided insights to the breeder

on the important farmer-preferred traits to incorporate when breeding maize for FAW resistance in SSA. The differences in FAW damage severity between the surveyed districts for the 2016 and 2017 cropping seasons suggest that the FAW is building stable populations with successive seasons across all regions in SSA. Further, identification of FAW resistance as a key trait to be considered in maize cultivar development in Zambia emphasized the need for initiating dedicated breeding programs targeting FAW and other identified farmer-preferred traits such as drought tolerance.

Germplasm development

Genetic variation is a pre-requisite for any successful breeding program. Before the arrival of FAW, maize breeding programs in SSA focused on improving insect resistance for some targeted insect pests including *Busseola fusca* Fuller (Lepidoptera: Noctuidae), *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), *Eldana saccharina* Walker (Lepidoptera: Pyralidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). With FAW being a new pest in SSA, screening for genetic variation in locally-adapted maize germplasm was essential for breeding. The significant variation for FAW resistance and important agronomic traits exhibited by the tropical inbred lines, and the identification of promising genotypes with partial FAW resistance, revealed the potential for developing maize cultivars with both stable resistance to FAW and a range of farmer-preferred traits. Stable resistance is especially important for the SSA environment because the prevailing climatic conditions support multiple annual generations of FAW, which accelerates pest evolution. Identification of the key traits contributing to ≥80% of the variation in the current population, such as anthesis-silking interval, plant height, FAW leaf and cob damage, desirable ear aspect and grain yield, provided further insights into the nature of the germplasm and the possibility of trait improvement. The negative correlation between grain yield and FAW-related traits suggested that FAW damage to maize reduces grain yield, raising the need for research to quantify FAW-associated yield losses across SSA. This would quantify the scale of losses to FAW problem and its impact on food security and livelihoods in SSA. The identified genotypes with partial FAW resistance and other favourable agronomic traits, included CZL1310c, CML444-B, CZL15220, TL1512847 and CML491, which can provide a foundation for FAW pre-breeding and breeding initiatives targeting SSA agroecologies.

Rearing of FAW and artificial screening of maize genotypes for FAW resistance

Development of comprehensive integrated pest management strategies (IPM) requires a good knowledge of the pest's biology, growth and development, and specialized facilities for the

artificial rearing of the pest under controlled conditions. This approach provides useful insights on pest behaviour and pest-plant interactions for target environments. The study on the growth and development of FAW achieved in this research provided clues on pest control such as the most vulnerable FAW growth stages to target when incorporating some recommended control practices, and in the application of IPM strategies to FAW management. For instance, the study found that the most fragile stage and the one with the highest mortality for FAW was during the pupal stage. This guides the need to focus scheduled planting and application of control measures against FAW at the pupal stage. Differential reactions to FAW exhibited by the tested maize genotypes with laboratory-reared FAW indicated the potential for effective germplasm screening and validation of field-observed FAW resistance using artificial inoculation techniques. The identified genotypes with partial seedling and whorl-stage foliar resistance to FAW included TL13159, TL02562, TL142151, VL050120, CML548-B, CML545-B, CZL1310c, CZL16095, EBL169550, and ZM 4236. These are useful genetic resources for FAW pre-breeding and breeding programs.

Genetic diversity of maize populations

The success of a breeding program is measured by the rate of adoption of resulting varieties by farmers and the rate of adoption is determined by the incorporation of farmer-preferred traits in the new varieties, among others. Farmers' varieties are adapted to local farming conditions and circumstances, and possess traits preferred by the farmers. Therefore, assessing the genetic diversity within locally-adapted germplasm is important to enable their subsequent incorporation in a breeding program. The high significant genetic variation among the genotypes is promising for improving grain yield, ear position, and FAW leaf and cob damage resistance using landraces. Higher levels of genetic variation were detected within the population than between the population by SNPs markers, implying that improving the identified traits would be best achieved using parental genotypes drawn from a common population. The three best OPVs, namely Pool 16, ZM 4236 and ZM 7114, should be incorporated in breeding maize for improved FAW resistance and farmer-preferred agronomic traits.

Combining ability and gene action

Understanding the genetic basis of inheritance for target traits is important for determining the most appropriate breeding strategies that will increase gains in selection. Combining ability and variance component analysis among promising lines are prerequisites to identify superior genetic material and to deduce gene action. The positive significance of both general and

specific combining ability effects suggested that both additive and non-additive gene action play an important role in the inheritance of FAW related and agro-morphological traits. The preponderance of non-additive genetic effects for FAW resistance and grain yield suggests that heterosis breeding is the best approach for improving these traits in the current population. The selected experimental hybrids with promising FAW resistance and agro morphological traits including CML346/EBL16469, ZM4236/CML545-B, CML346/CZL1310c, CML334/EBL173782 and CML545-B/EBL169550, and can be used as hybrids for continued FAW resistance breeding in Zambia. These hybrids can be evaluated in multiple locations and advanced through three or four generations. Recurrent selection methods can be applied to harness partial FAW resistance through:

- OPV development- to exploit the additive genetic effects, targeting mainly the smallholder farmers;
- Inbred line development leading to hybrid breeding- to exploit the non-additive genetic effects, targeting mostly the commercial farmers;

Future research could also explore the prospect of multi-line variety development because of the variable resistance mechanisms associated with reduced FAW cob damage, and the presence of early- and mid-whorl FAW resistance revealed in the study. Multi-line varieties would create a more durable form of resistance by increasing the heterogeneity of the germplasm and broadening the genetic base of the new cultivars.